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The Administration of Flumethasone, by three Different Routes, its Measurement in the Plasma and Some Effects on 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

Flumethasone was given to Merino wethers weighing 30–50 kg at rates of 0·62–1·35 mg/kg$^{0·75}$ by intravenous (experiments 1 and 2), intraruminal (experiment 4) and subcutaneous (experiment 5) routes over 8 days. In experiment 3, 1·2 mg flumethasone/kg$^{0·75}$ was given intravenously over 4, 5 or 6 days. The plasma concentration profiles showed concentrations in the order: intravenous > subcutaneous > intraruminal. Plasma concentration patterns usually were highest during the first 48 h of infusion followed by relatively stable values. This last feature was not evident in experiments when the rate of hormone infusion was increased. Estimates of the metabolic clearance rates for flumethasone in experiments 1, 2 and 5 were 200–700 ml/min during the equilibrium concentration periods.

The effects of flumethasone on some aspects of wool growth revealed interactions between the routes of administration, the period of dosage and the rate of wool growth in the recipients.

In experiments 1 and 2 intravenous infusion of 1·20–1·33 mg flumethasone/kg$^{0·75}$ caused the shedding of all wool fibres about 30 days after treatment. Some effects of dosing sheep with flumethasone at a time when wool growth was decreasing were also observed in experiment 2. Flumethasone given at a rate of 1·2 mg/kg$^{0·75}$ over 4, 5 or 6 days caused the shedding of only some wool fibres which were firmly retained on the sheep by the continuous fibres. Intraruminal and subcutaneous infusions of 0·62–1·35 mg flumethasone/kg$^{0·75}$ had similar results to the last in the majority of animals although in a few cases no discontinuity of wool fibres was observed.

Recovery in wool growth was observed after treatment. Animals regained their pretreatment wool growth in experiments 1, 4 and 5 by 60 days after treatment and probably equalled at that time wool growth in controls. Recovery was retarded in some individuals in experiment 2 and in some groups in experiment 3. In experiment 1, 21 days wool growth was estimated to have been lost.

Some aspects of complete versus partial shedding of wool fibres are discussed particularly with reference to wool harvesting. Some similarities in the appearance of fleeces of steroid-treated sheep and naturally shedding animals are also discussed.

In some experiments, particularly when the infusion rate of flumethasone was increased (experiment 3), the sheep showed temporary but significant feed refusals during, but more commonly after, treatment. Speculative discussion as to the metabolic causes of this response is included.

Introduction

Removal of wool from sheep by inducing fibre shedding with chemicals is being studied as a possible alternative to conventional shearing. Early observations on the inhibitory effects of exogenously administered adrenal glucocorticoids on growing hairs during hair growth cycles in rats (for a review see Mohn 1958) were followed by reports on similar effects of cortisone acetate on wool fibres in Merino sheep (Lindner and Ferguson 1956). Since then some effects on wool growth in sheep of a small number of analogues of cortisol have been studied (Ferguson *et al.* 1965; Panaretto *et al.* 1975; Panaretto and Wallace 1978).

The gradual development of analogues more powerful than the natural hormones, but without undesirable side effects, has been described by Sarett (1959). Briefly, the unsaturation of the C_1 double bond and the inclusion of fluorine in the α configuration in the 9 position of the steroid molecule greatly enhanced anti-inflammatory activity by reducing the enzymic reduction of ring A. The 9 α-halogen also inhibited reduction of the ketone group at position 20 and cleavage of the side chain at position 17 (Florini *et al.* 1961). Compounds such as 9-fluorohydrocortisone could not be used until the inclusion in the molecule of a 16 α-methyl group overcame their salt retention properties.

Dexamethasone [9-fluoro-11,17,21-trihydroxy-16α-methyl-pregna-1,4-diene-3,20-dione] and betamethasone (same structure as dexamethasone except for the β configuration of the 16 methyl group) have greatly enhanced anti-inflammatory properties when compared to cortisol. Unlike cortisol, which is metabolized in sheep mainly by A ring reduction (Lindner 1972), the two analogues are metabolized in human beings and the rat mainly in 6, 11, 20 and 17 positions (Butler and Gray 1970; Rice *et al.* 1974). Butler and Gray (1970) stated that the metabolism of betamethasone in human beings is mainly by the relatively slow reactions of 6β-hydroxylation and reduction of the ketone at C-20.

Panaretto and Wallace (1978) found what appeared to be a steadily increasing disappearance rate of dexamethasone from the plasma of sheep during the last 6 days of an 8-day infusion.

In this paper we were concerned with the metabolism and effects of flumethasone [6-9-fluoro-11,17,21-trihydroxy-16α-methyl-pregna-1,4-diene-3,20-dione] given intravenously, intraruminally and subcutaneously on the wool growth of sheep, our interest in the molecule arising primarily from the fluorine at position 6 in the molecule; this structure might conceivably retard the rate of metabolism of the compound and so give more uniform effects with respect to inhibiting wool growth than dexamethasone.

Materials and Methods

The Sheep, their Housing and Nutrition

The Merino wethers used in experiments 1, 2 and 3 were adult and those used in all other experiments were approximately 1·5 years old at the time of treatment. Each animal was housed in a cage in an animal house and offered 600 g of a mixture of equal parts of lucerne chaff and oats daily. The sheep in experiment 2 were given 1000 g of the same feed mixture 14 days after the infusion finished. All feeds were given at 1600 h.

The body weights of the sheep included the weight of wool (*c.* $\geqslant$ 5 cm staple); body weights raised to the power 0·75 were used in calculating dosages of hormones.

Infusions

Flumethasone infusate

Each millilitre of stock flumethasone solution (Flucort^R solution, Diamond Laboratories, Des Moines, Iowa, U.S.A.) contained 0·5 mg flumethasone, 420 mg polyethylene glycol 400, 9 mg benzyl alcohol, 8 mg sodium chloride and 0·1 mg citric acid. Appropriate quantities of stock solution (see Table 1) were mixed with 0·9% (w/v) sodium chloride to give a final volume of 45 ml for infusion over 24 h. The dose rates and routes of administration of flumethasone are given in Table 1.

Vehicle infusate

Appropriately diluted vehicle was infused into control animals in some experiments (see below).

Infusion technique and blood sampling

Infusions were made using Harvard infusion pumps (series 932 Harvard Apparatus Co. Inc.

Mass., U.S.A.) and through polyethylene tubing i.d. 0·86 mm, o.d. 1·52 mm (Dural Plastics Pty Ltd, Dural, N.S.W.). Pumping was continuous at a rate slightly less than 2 ml/h for the periods shown in Table 1. Intravenous infusions were made into the jugular vein, subcutaneous infusions were made by implanting 10 cm of tubing subcutaneously in the dorsal cervical region approximately overlying the first three cervical vertebrae, and intraruminal infusions were made via ruminal cannulae which had been inserted one month before experimentation.

All blood samples were obtained by jugular venipuncture at 0900 h and in some cases at 1500 h; in some cases, usually on the first or last days of infusion, blood samples were taken more frequently. Separated plasma was stored at −20°C until analysis.

Wool Growth

Care was taken to ensure that the ranges of wool growth in control and treated sheep were comparable during the control period. Wool growth was measured in each animal by shearing the wool from defined areas of 100 cm^2 on the mid-side of each sheep. Each sample was conditioned and weighed. The clean dry weights were measured using the method of Hemsley *et al.* (1973). Control values in the experiments were the means of at least six clippings during the 12 weeks immediately before treatment.

The effects of flumethasone on wool growth fell into three distinct categories: (1) shedding of the fleece was defined as the state where *all* wool fibres were shed and lay outside the fibre canal so that the fleece fell from the animal (those cases where shedding was confined to restricted body regions have received specific mention), (2) no shedding or discontinuity of wool fibres, and (3) the fleece was not cast but contained a greater or lesser proportion of shed or discontinuous fibres among the continuous ones that retained the fleece as a whole on the animal. The last fibres were still growing and so carried the shed fibres from the surface of the skin. After several weeks they appeared as a distinct zone connecting the outer wool, which was predominantly shed fibres, to the skin where new fibres were emerging. The distribution of shed fibres frequently varied between regions of the body of an individual (see below) and because of this no effort was made to quantify the response. Subjective estimates of shedding which have been given in a few cases below are intended to convey the magnitude of the proportion of broken fibres on *all* body regions.

Analytical Methods

Plasma cortisol

Plasma cortisol concentrations were measured using the method of Bassett and Hinks (1969).

Metabolic clearance rates (MCR)

MCR values expressed the volume of blood cleared of the specified molecule per unit time and were calculated by dividing the infusion rates of the molecule by appropriate *equilibrium* concentrations in plasma where these were observed in experiments 1, 2 and 5.

Flumethasone

Flumethasone was assayed using the radioimmunoassay system described for dexamethasone by Panaretto and Wallace (1978) and derived from the method of Hichens and Hogans (1974). In the present system flumethasone was able to displace labelled dexamethasone from the antiserum as actively as dexamethasone.

Standards were prepared in pooled ovine plasma after the method of Hichens and Hogans (1974). This procedure should ensure that recoveries in unknowns were equal to those in standards provided conditions in pooled plasma equalled the experimental samples. This supposition was tested by comparing standard curves made up in pooled plasma with those made up in plasma from three animals that had never had steroid analogue treatment (see below). The assay curve was on a scale 0, 0·25, 0·5, 1, 2, 4, 8 and 16 ng/ml, each concentration being assayed in triplicate. Examination of the standard curve (counts bound on an arithmetic scale *v.* concentration on a logarithmic scale) revealed that the limit of sensitivity of the method was 1 ng/ml. Thus, all values < 1 ng/ml were equated to zero. In two cases pretreatment values of > 1 ng/ml were recorded (Figs 3 and 7) but were not investigated further. Cross-reactions between label and plasma were measured by including samples of pre-infusion plasma and label ['no antibody' control (Hichens and Hogans 1974)] with each assay; the mean counts bound here were subtracted from assay unknowns.

Comparisons of the mean counts bound between triplicates within standard curve assays yielded coefficients of variation (c.v.) in the range 2·6–5·4%. The c.v. between the four means for each locus

on the four standard curves was in the range 4–9%. In intra-assay c.v. values for plasma concentrations (pc) of [$1 \leqslant pc \leqslant 2$], [$2 < pc \leqslant 8$], [$8 < pc \leqslant 16$], and > 16 μl/ml in four successive assays were 6·3 ($n = 21$), 3·3 ($n = 40$), 3·7 ($n = 7$) and 2·0% ($n = 5$) respectively.

Table 1. Experimental treatments and flumethasone dosage regimes and rates for sheep in experiments 1–5

i.v., Intravenous; i.r., intraruminal; s.c., subcutaneous

Expt No.	Treatment	Duration of treatment (days)	Sheep No.	Body weight (mean ± s.e.m.) (kg)	(kg$^{0.75}$)	Flumethasone (mg)	(mg/kg)	(mg/kg$^{0.75}$)
1	Uninfused controls		2253, 2259, 2260, 2268	40·7 ±0·7	16·1			
	Vehicle infused i.v.	8	2247	32·6	14·3			
			2264	36·6				
	Flumethasone i.v.	8	2263, 2262, 2258	40·2 ±0·3	16·0	19·6	0·49	1·2
2	Controls		5019	49·7	18·5			
			5036	48·5				
	Flumethasone i.v.	8	5125	41·0	16·5	22	0·52	1·33
			4976	43·0				
	Flumethasone i.v.	8	5087	45·8	17·7	12	0·26	0·68
			5099	46·1				
3	Flumethasone i.v.	4	2252	37·6	15·3	18·7	0·49	1·2
			2267	38·6				
	Flumethasone i.v.	5	2254	34·2	14·3	17·7	0·51	1·2
			2256	35·2				
	Flumethasone i.v.	6	2245	42·2	16·6	20·4	0·48	1·2
			2246	42·3				
4	Flumethasone i.r.	8	2248	45·4	17·6	10·9	0·24	0·62
			2250	46·2				
			2251	47·0	49·4			
			2255	49·6	±1·3 18·6	23·6	0·48	1·27
			2265	51·6				
5	Uninfused controls		8 sheep	31·9 ±0·9	13·4			
	Flumethasone s.c.	8	1265	35·8				
			1267	28·2	32·1	13·5	8·9	0·27	0·66
			1383	33·3	±1·6			
			1615	31·1				
		8	1508	36·8	33·4			
			1887	33·4	±1·9 13·9	18·8	0·56	1·35
			1570	30·1				

The specificity of the antiserum was similar to that described by Hichens and Hogans (1974). Endogenous cortisol could not have reacted with the antiserum (see Fig. 8). Other endogenous hormones in castrate males were probably present in very low amounts. The most likely cross-reactors, metabolites of flumethasone, are presently not known and we were unable to test for this.

Experimental Procedure

The experiments performed are given in Table 1. Basically, some aspects of the interactions between doses of flumethasone, routes and times of administration, and nutrition on wool growth were examined.

Experiments 1, 4 and 5 examined the effects of administering the analogue to sheep by three routes: intravenously, intraruminally and subcutaneously respectively. Experiment 2 had two main features: first, wool growth was decreasing in all animals at the time of treatment with flumethasone and, secondly, the quantity of feed given was increased to 1000 g daily 14 days after treatment. Experiment 3 examined the effects on a number of parameters of decreasing the period of intravenously infusing flumethasone.

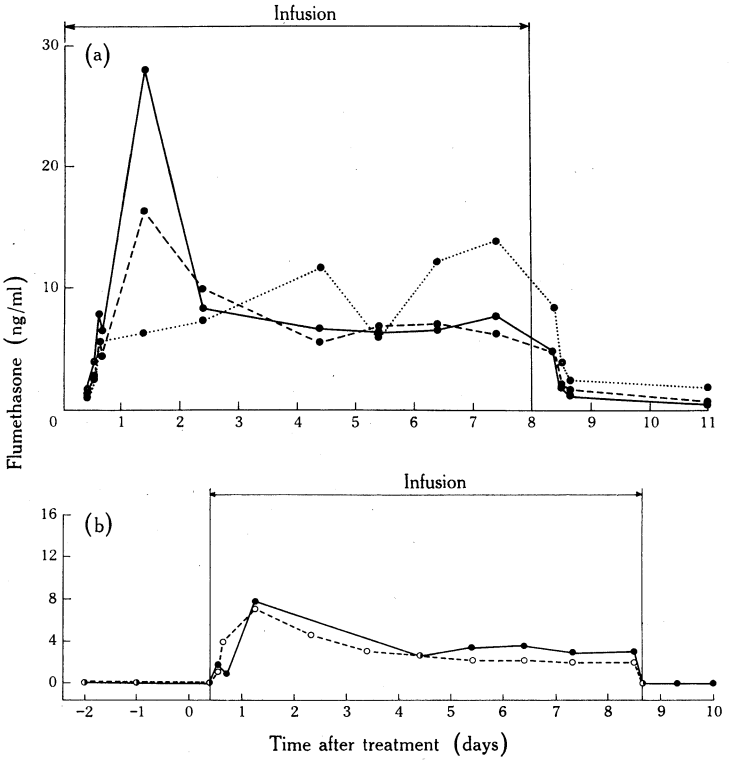

Fig. 1. Plasma flumethasone concentrations in sheep in (*a*) experiment 1 and (*b*) experiment 2. Flumethasone was infused intravenously at a rate of 1·2–1·4 mg/kg$^{0·75}$ during the 8 days indicated.

Results

Experiment 1, *Intravenous Infusion with Dietary Restriction*

Plasma flumethasone concentrations and MCR values

No flumethasone was detected in the plasma of control sheep or infused sheep before flumethasone infusions were made. Plasma flumethasone concentrations of sheep dosed intravenously for 8 days at the rate of 1·2 mg/kg$^{0·75}$ are illustrated in Fig. 1*a*. In two animals, 2262 and 2263, maximal flumethasone concentrations were reached during the first 48 h of infusion and relatively stable concentrations in the range 6–8 ng/ml were then measured. Sheep 2258 showed a relatively stable plasma

flumethasone concentration of 6–12 ng/ml throughout infusion. The MCR for flumethasone in these experiments approximated 200–300 ml/min assuming equilibrium flumethasone concentrations of 7 ng/ml for two of the sheep and 9–10 ng/ml for the third (Fig. 1).

Wool growth

Wool growth was measured for 12 weeks before experimental treatments were applied. The quantities of clean dry wool harvested from mid-side patches are illustrated in Fig. 2*a*. There appeared to be no effect of infusing vehicle on wool growth in sheep. Intravenous infusion of flumethasone depressed wool growth at day 20 to a mean ± s.e.m. of 21 ± 5% of pretreatment values (see Fig. 2*a*). Wool growth then slowly recovered so that it was equal to that in controls approximately 62 days after infusion.

All three sheep given 1·2 mg flumethasone/kg$^{0·75}$ intravenously completely shed their fleeces approximately 30 days after infusion; 3·2–6·0 kg wool was shed. Wool production lost due to flumethasone treatment was approximately equivalent to 21 days wool growth.

Food eaten

Control sheep ate all their rations. Flumethasone treatment, however, frequently resulted in feed refusals during the infusion period, but more commonly after it. These data are best exemplified by the result in experiment 3 (see below).

Only one flumethasone-treated animal refused food in experiment 1; sheep 2258 left 58, 30 and 10% of its ration on days 3, 4 and 5 respectively after infusion.

Experiment 2, *Intravenous Infusion and Dietary Increase*

Plasma flumethasone concentrations and MCR values

Plasma flumethasone concentrations in sheep 5125 and 4976 are illustrated in Fig. 1*b*. In all sheep in this experiment peak flumethasone concentrations were again recorded during the first 48 h of infusion and these tended to be followed by relatively stable concentrations of 2–4 and 1–2 ng/ml in the animals dosed at 1·33 and 0·68 mg flumethasone/kg$^{0·75}$ respectively. MCR values for flumethasone in these animals were approximately 650–700 ml/min assuming equilibrium flumethasone concentrations of 3·0 and 1·5 ng/ml respectively (Fig. 1).

Wool growth

Wool growth measurements were unique in these animals and are illustrated in Figs 2*b* and 2*c*. During the 14-week pretreatment period wool growth increased and then was decreasing for 4–6 weeks before infusion. This fall in wool growth continued in the control animals and did not start to recover until 14 days after infusion. Wool growth then increased in all animals though very much more slowly in sheep 5099. Wool harvested from tattooed patches in two sheep treated with 1·4 mg flumethasone/kg$^{0·75}$ had values of 7·5 and 7·6% of mean pretreatment values on day 14 after infusion and values of 45 and 46% respectively on day 28. These animals shed their fleeces about one month after infusion, 3·3 kg of wool being removed from each sheep at that time.

Wool could not be removed in the same way 30 days after treatment from either animal given 0·68 mg flumethasone/kg$^{0·75}$. Wool growth on tattooed patches in

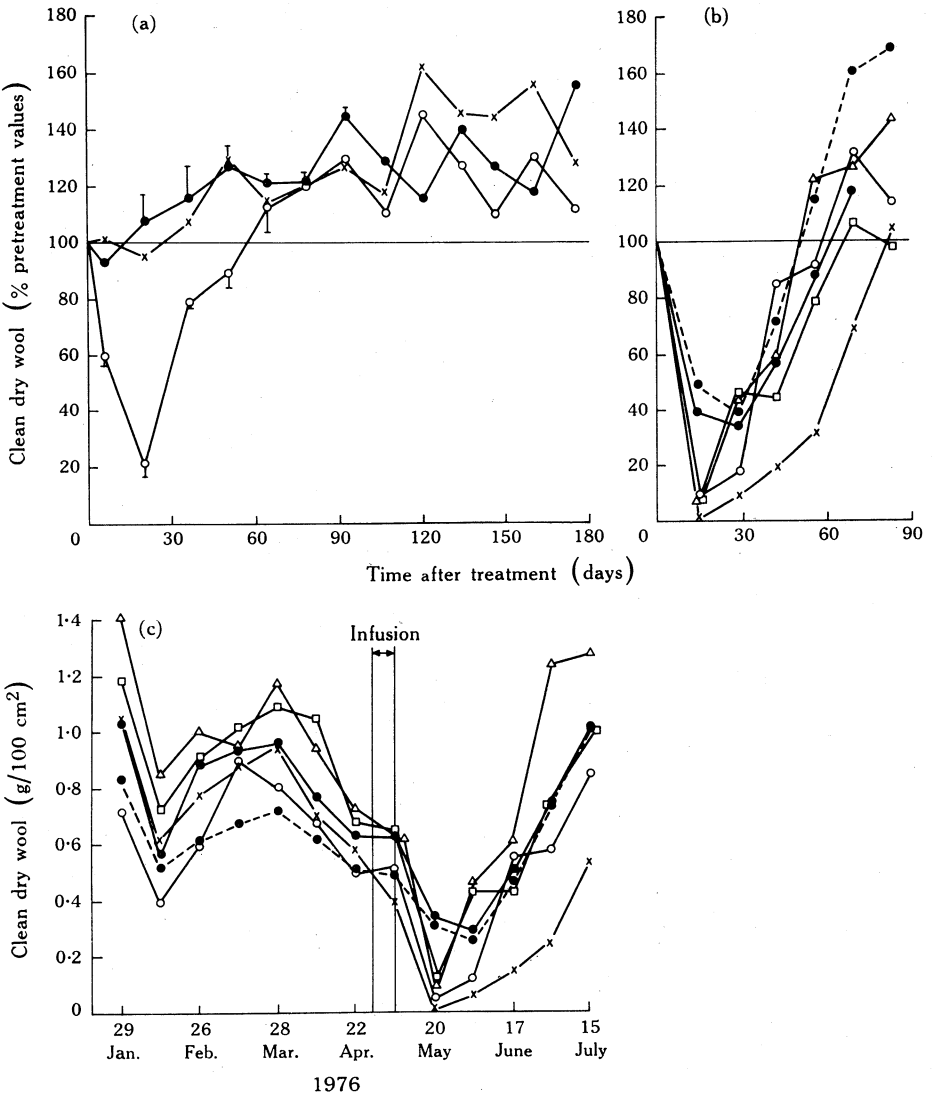

Fig. 2. Clean dry wool harvested from patches of 100 cm² marked on the mid-side regions of sheep in experiments 1 and 2. Days indicate the time after infusing flumethasone intravenously, at the rate of 0·68 or 1·2–1·35 mg/kg$^{0.75}$ body weight (see Table 1). Ordinal values represent mean quantities of clean dry wool harvested expressed as a percentage of values obtained before infusions started. Standard errors of the mean are represented by vertical lines. (*a*) Experiment 1. ● Controls ($n = 4$). × Vehicle-infused controls ($n = 2$). ○ Flumethasone-treated sheep ($n = 3$). (*b*) and (*c*) Dry clean wool harvested from a patch of 100 cm² on the mid-side region of sheep used in experiment 2 expressed as a percentage of pretreatment values (Fig. 2*b*) and expressed as a weight (Fig. 2*c*). The animals received 600 g of a mixture of lucerne chaff and oats (1 : 1) until 20 May 1976 and 1000 g of this same mixture thereafter. The symbols used here are: control sheep 5019 (●– – –●) and 5036 (●——●); treated sheep (0·68 mg/kg$^{0.75}$) 5099 (×) and 5087 (○); treated sheep (1·44 mg/kg$^{0.75}$) 4976 (△) and 5125 (□).

these animals was 0 and 9·2% on days 14 and 28 after infusion in sheep 5099, and 8·5 and 17·4% on days 14 and 28 after infusion in sheep 5087 (see Fig. 2*c*). On day 43 after infusion 2·7 kg wool was shed from a substantial part of the body of sheep 5087; however, wool was not cast from the cervical and anterior thoracic regions of the animal even though enough fibres were shed to constitute an evident 'break' in the fleece. Regrowth was so retarded in sheep 5099 that shedding of the fleece was not recorded until day 67 after infusion.

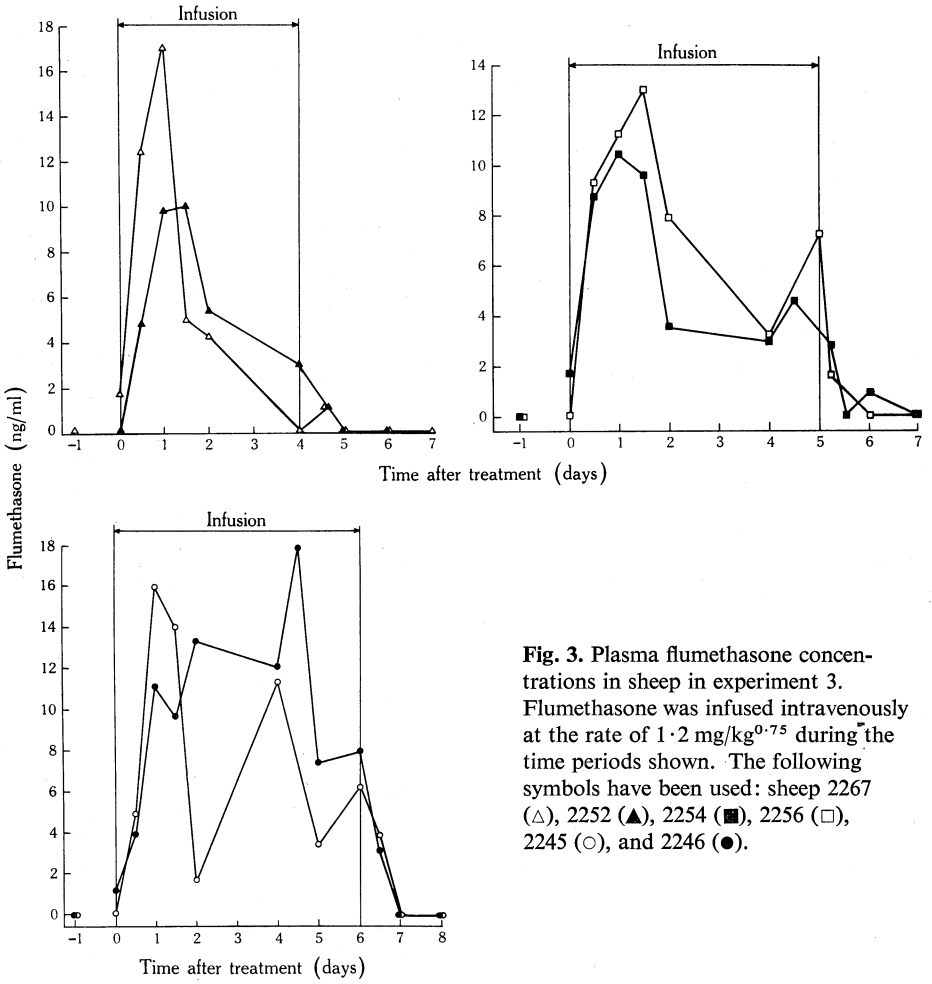

Fig. 3. Plasma flumethasone concentrations in sheep in experiment 3. Flumethasone was infused intravenously at the rate of 1·2 mg/kg$^{0·75}$ during the time periods shown. The following symbols have been used: sheep 2267 (△), 2252 (▲), 2254 (■), 2256 (□), 2245 (○), and 2246 (●).

Food eaten

In experiment 2, sheep 5125 (1·4 mg flumethasone/kg$^{0·75}$) refused 30, 90, 64 and 73% of its ration on the first 4 days of infusion, sheep 5099 (0·68 mg flumethasone/kg$^{0·75}$) refused 29, 78 and 76% of food given on days 3, 4 and 5 respectively of infusion while sheep 5087 (same treatment as 5099) refused 10 and 58% of its food on the first and second days after infusion. No further rejections of food were recorded even when the quantity of feed was increased to 1000 g.

Experiment 3, *Intravenous Infusion over a Variable Time*

Plasma flumethasone concentration and MCR values

Plasma flumethasone concentrations (Fig. 3) were at their highest, 10–17 ng/ml, during the first 48 h infusion and tended to decrease subsequently. There were, however, exceptions to this: sheep 2245 showed an initial peak in concentration during the first 48 h of infusion followed by a second peak at 96 h, and sheep 2246 showed persistently high values in the range 10–18 ng/ml throughout infusion.

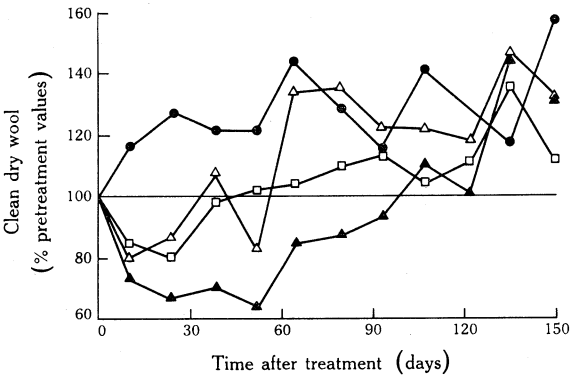

Fig. 4. Clean dry wool harvested from patches of 100 cm^2 delineated on the mid-sides of sheep in experiment 3. Days indicate the time after cessation of intravenous infusion of flumethasone at the rate of 1·2 mg/kg$^{0·75}$ over 4 (▲), 5 (□) and 6 (△) days. Ordinal values represent the quantities of wool removed expressed as a percentage of control values obtained before infusions were made. Control sheep (●, $n = 4$) represent values from a group of uninfused control animals.

Wool growth

Experiments 3–5. None of the sheep completely shed its whole fleece when the period of intravenous administration of flumethasone was shortened (experiment 3) or when the intraruminal (experiment 4) or subcutaneous (experiment 5) routes were used. Two principal variants were seen in these animals: first, differences between animals and second, differences between body regions in an individual. Between sheep differences were exemplified by the fact that at the same dose rate of flumethasone a minority of animals showed little effect on wool growth while in others the proportion of wool fibres shed was great enough to constitute an easily discernible 'wool break' (for an example see experiment 3 below). In any individual the proportion of shed or 'broken' fibres tended to be most marked on the belly and ventral body regions than on the dorsum. The least evident degree of 'broken' fibres was always in the cephalic and cervical regions of any animal. For example sheep 1570 (experiment 5) shed the wool from its belly and lateral surfaces of its forelegs about one month after treatment while the 'broken' wool fibres over the other body regions were retained *on* the animal by the unbroken fibres still remaining.

Experiment 3. The amounts of clean dry wool removed from mid-side tattooed patches of these experimental animals, expressed as percentages of control values, are illustrated in Fig. 4. Wool growth was depressed in the treated animals but the degree of depression was maximal at 60% of an animal's own pretreatment value in one of the 4-day treated sheep. The degree of wool growth depression was even greater relative to untreated controls. Another feature of these results was the stratification of wool growth response; in general the animals infused with flumethasone at the greatest *rate* had greatest wool growth depression while those infused

at lesser rates, i.e. over longer periods, were stratified above this according to dosage rate.

Recovery in wool growth was gradual and while sheep that had been infused for 5 or 6 days were growing an amount of wool equal to that during the control period by the 38th day following infusion those infused for 4 days did not achieve this until approximately 100 days after infusion. At approximately 66–108 days after infusion all treated animals were growing as much wool as untreated controls.

One month after infusions finished there were two extreme responses. Sheep 2252 (1·2 mg flumethasone/kg$^{0·75}$ administered over 4 days) showed very evident discontinuity in the majority of its wool fibres but still retained its fleece, while sheep 2256 (same dose of flumethasone given over 5 days) showed no discontinuity of its wool fibres at all. Other sheep lay between these extremes.

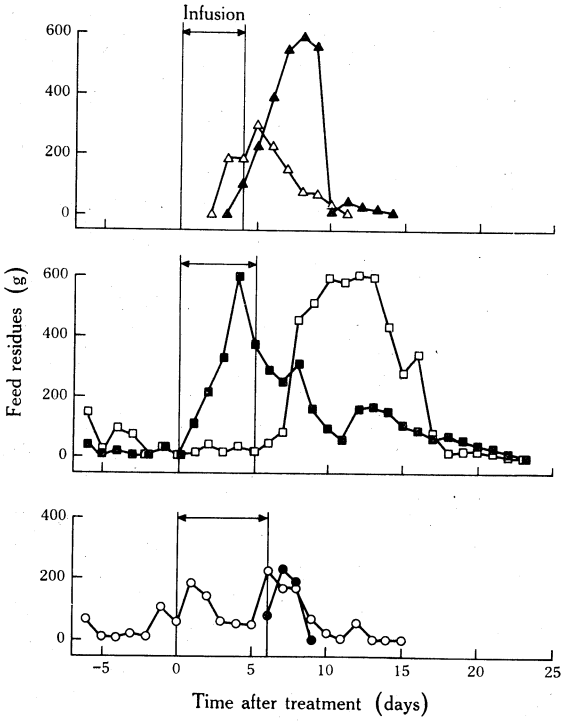

Fig. 5. Feed residues in sheep in experiment 3 before, during and after they were infused intravenously 1·2 mg flumethasone/kg$^{0·75}$. Each sheep was offered 600 g of a mixture of lucerne chaff and oats (1 : 1). The following symbols have been used: sheep 2267 (△), 2252 (▲), 2254 (■), 2256 (□), 2245 (○), and 2246 (●).

Food eaten

The significant feed refusals in these animals are illustrated in Fig. 5; control animals left no feed during the periods illustrated. The feed refusals occurred either during infusion or in the period following it.

Experiment 4, *Intraruminal Infusions*

Plasma flumethasone concentrations

Mean plasma flumethasone concentrations, < 1 ng/ml, lay at the lower part of the sensitivity of the assay method during the intraruminal infusion of flumethasone.

Wool growth

Wool growth in these animals is shown in Fig. 6*a*. Animals treated intraruminally with 1·27 mg flumethasone/kg$^{0·75}$ had recovered their wool growth by day 57 post-infusion while those treated at half this dosage rate appeared to take somewhat longer. Wool growth in animals treated at the higher dose rate was not different from controls by day 57.

Two animals showed a similar degree of wool growth depression to the i.v.-infused animals in experiment 1, whilst wool growth in three of the animals resembled the response seen in experiment 3. None of the animals shed its fleece and again variation in response approximately one month after infusion lay between a discontinuity in the major number of fibres in sheep 2250 and no broken fibres in sheep 2265. The responses of other animals were between these extremes.

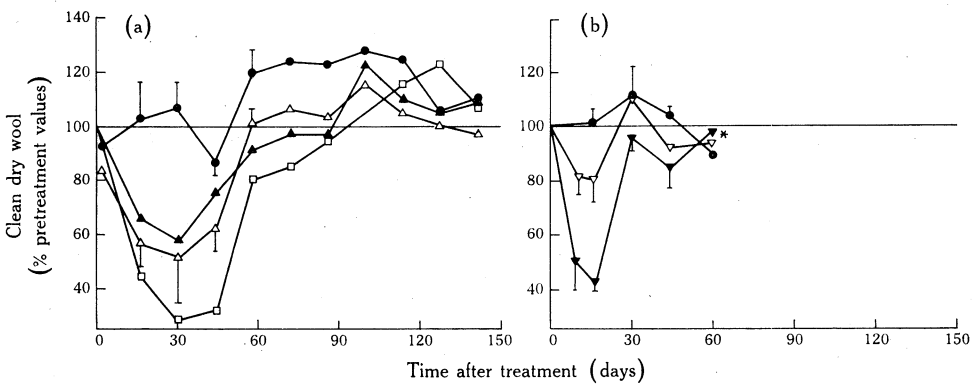

Fig. 6. Clean dry wool harvested from patches of 100 cm^2 on the mid-side region of sheep in experiments 4 and 5. Days indicate the time after infusing flumethasone (*a*) intraruminally and (*b*) subcutaneously at the rate of 0·66–1·35 mg/kg$^{0·75}$ (see Table 1). Ordinal values express the mean quantities of wool harvested as a percentage of pretreatment values. Standard errors of the mean are represented by vertical lines. (*a*) Experiment 4. ● Controls (n = 4). △ Sheep treated with 1·27 mg flumethasone/kg$^{0·75}$ (n = 3). Two sheep were given 0·62 mg flumethasone/kg$^{0·75}$: sheep 2248 (▲) and 2250 (□). (*b*) Experiment 5. ● Uninfused controls (n = 8). Flumethasone-treated sheep: 0·68 (▽) and 1·35 (▼) mg/kg$^{0·75}$ (see Table 1). Clipping of tattooed patches which should have occurred at the times marked by an asterisk were actually made 2 weeks later, thus the values at 58 days were divided by two in order to obtain those for 44 days.

Food eaten

Feed refusals were detected in only two sheep. Sheep 2248 refused 50% of feed offered on the last day of infusion and 58 and 42% on days 1 and 3 respectively following infusion; sheep 2265 refused 30% of its feed on the day following infusion.

Experiment 5, Subcutaneous Infusions

Plasma flumethasone concentrations and MCR values

Plasma flumethasone concentrations measured during the subcutaneous infusion of the hormonal analogue are illustrated in Fig. 7.

At the lower dose rate (0·66 mg flumethasone/kg$^{0·75}$) plasma flumethasone concentrations were relatively stable throughout the infusion in the range 1·5–4·5 ng/ml. MCR values for the three animals, assuming a mean flumethasone concen-

tration of approximately 2 ng/ml, were approximately 400 ml/min and one-half this value was found in animal 1615 with approximately 4·5 ng flumethasone/ml.

In those animals that received the greater dose of flumethasone, 1·35 mg/kg$^{0·75}$, two sheep (1570 and 1508) showed greater concentrations during the first 48 h of

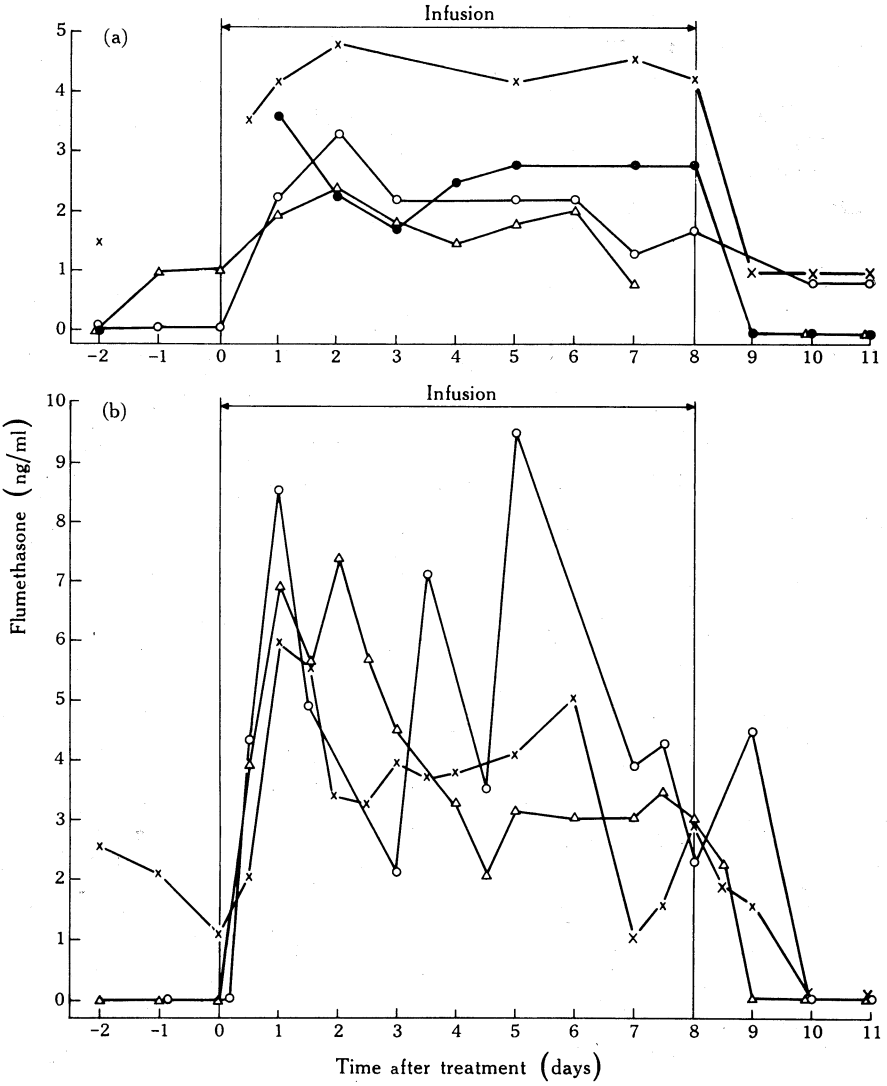

Fig. 7. Plasma flumethasone concentrations in sheep in experiment 5. Flumethasone was infused subcutaneously at the rate of (*a*) 0·66 and (*b*) 1·35 mg/kg$^{0·75}$ during the time period indicated. The following symbols have been used: in (*a*) sheep 1265 (△), 1267 (○), 1383 (●), and 1615 (×); in (*b*) sheep 1508 (×), 1570 (△), and 1887 (○).

infusion than subsequently when both animals tended to have values of 3–4 ng/ml. The MCR value during this period was about 450 ml/min assuming an equilibrium concentration of 3·5 ng/ml. Sheep 1887 showed large fluctuations in plasma flumethasone concentrations throughout infusion.

Sheep 1570 and 1508 showed shedding in approximately 75% of their fibres while sheep 1887 showed less shedding than this. Taken as a whole the proportion of shed fibres was less in the animals on the lower flumethasone dose; however, there were no shed fibres visible in sheep 1615 which had the highest plasma flumethasone concentrations in the group.

Wool growth

The wool growth responses of infusing flumethasone subcutaneously are illustrated in Fig. 6*b*. The mean depression in wool growth was dose-dependent (20 and 40% of pretreatment values for animals treated with 0·68 and 1·35 mg/kg$^{0·75}$ respectively). Wool growth in both groups appears to have recovered, relative to their own pretreatment values and untreated controls, by day 44 postinfusion.

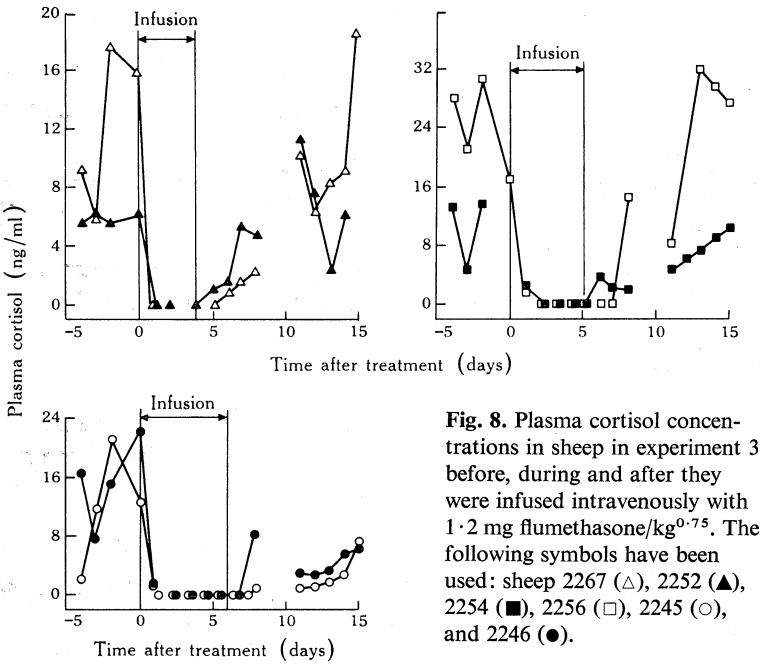

Fig. 8. Plasma cortisol concentrations in sheep in experiment 3 before, during and after they were infused intravenously with 1·2 mg flumethasone/kg$^{0·75}$. The following symbols have been used: sheep 2267 (△), 2252 (▲), 2254 (■), 2256 (□), 2245 (○), and 2246 (●).

Responses in terms of fleece shedding were similar to those seen in experiment 4. At the lower dose rate no discontinuity could be discerned in the fibres of one animal but approximately 50% of wool fibres were estimated to be discontinuous in the animal showing the greatest effect. In the animals dosed at 1·35 mg/kg$^{0·75}$ a somewhat similar range of responses was seen except that approximately 75% of fibres were discontinuous in the two animals showing the greatest response.

None of these animals shed its fleece and the end result was very similar to that in experiment 4. The range of responses in those animals dosed at a rate of 0·66 mg/kg$^{0·75}$ was shown by sheep 1265 at one end in that approximately one-half its wool fibres were broken, and sheep 1615 at the other end in that no discontinuous wool fibres were evident. Similarly in those animals dosed at 1·35 mg/kg$^{0·75}$ the range of responses was from approximately three-quarters broken fibres (sheep 1570 and 1508) to fewer than one-half in sheep 1887.

Food eaten

Sheep 1570 left feed residues of 61, 58, 76, 52, 52·5 and 52% of the food offered on days 2–6 respectively following infusion.

Plasma cortisol concentrations

Uninfused controls and vehicle-infused control sheep showed no significant changes in plasma cortisol concentrations during an experiment. Mean values ranged from $6{\cdot}2 \pm 1{\cdot}5$ to $6{\cdot}9 \pm 0{\cdot}7$ ng/ml in those individuals at either end of the range.

In flumethasone infusion experiments plasma cortisol concentrations were sometimes elevated prior to infusion (see Fig. 8). No cortisol could be detected during the infusion period in *any* experiment and in some cases for 24–48 h after infusion finished. Recovery of plasma cortisol concentrations to equal those observed before the infusion were not always observed during an experimental period. These features were exemplified by sheep in experiment 3 (Fig. 8). In experiment 3 it can be seen that the pre-infusion values for plasma cortisol varied to reach levels of 20–30 ng/ml. Values during infusion were invariably below the level of detection of the analytical method. In some cases no cortisol was detected for 48 h following infusion (e.g. see sheep 2256, Fig. 8).

Recovery to pre-infusion levels was sometimes achieved within 3 days, e.g. sheep 2256 (Fig. 8) or in 7 days (although recovery may have occurred sooner), e.g. see sheep 2252 (Fig. 8).

In sheep 2245 and 2246 (Fig. 8) recovery was slower and plasma cortisol concentrations had probably not recovered completely 9 days after infusion.

Discussion

A number of variables appeared to influence the wool growth response of animals to flumethasone treatment and among these we have investigated the state of wool growth in the animals treated, the quantity and route of administration of the chemical, and the time period over which it was delivered.

The intravenous infusion of 1·2–1·4 mg flumethasone/$kg^{0{\cdot}75}$ over 8 days in experiments 1 and 2 resulted in complete fleece shedding in all five animals. In experiment 1 this was achieved at a plasma flumethasone concentration of 6–10 ng/ml and results obtained with subcutaneously administered flumethasone (experiment 5) might have indicated that this concentration was mandatory for this result. In the case of experiment 2, however, complete fleece shedding was obtained at much lower plasma flumethasone concentrations, namely 2–4 ng/ml. However, for reasons unknown, these animals had a declining wool growth rate at the time of treatment (Fig. 2*b*). Thus it might be suggested that much lower analogue concentrations can cause fleece shedding in such animals. This notion was supported by other evidence. For example, sheep 5087 ultimately shed a substantial part of its fleece at a relatively low dosage rate of flumethasone while wool growth in sheep 5099 was greatly depressed over a prolonged period, a result not encountered in any other sheep in these experiments.

Results obtained in experiments 4 and 5 stressed the fact that the route of administration of flumethasone was of paramount importance in affecting plasma concentrations. The intraruminal and subcutaneous routes of administration of flumethasone at dose rates of 1·2–1·35 mg/$kg^{0{\cdot}75}$ were quite ineffective in causing the complete

shedding of fleeces and indeed in some cases did not result in the shedding of a detectable number of wool fibres. These results when considered with respect to ruminal administration suggested significant symbiont modification of the flumethasone to metabolites ineffective in causing fleece shedding; this was emphasized by our inability to detect the hormone in concentrations greater than about 1 ng/ml plasma. An alternative or additional explanation is poor absorption of the analogue from the gut.

Not only is the wool growth rate in sheep at the time of treatment of importance in its effects on the outcome of steroid treatment, but the time period over which analogue was administered is also important. Results in experiment 3 indicated that the time period over which flumethasone was given could not be substantially shortened from 8 days. This was particularly emphasized by sheep 2246 where fleece shedding was not achieved even though a relatively high concentration of flumethasone was seen for 5 days of infusion. With respect to the need to sustain a desired concentration of hormone over a prolonged period it is of interest to note that Chang and Maibach (1967) and Singh and Hardy (1975) noted no regression of hair follicles in cultured pig and mouse skin respectively after 3 days of exposure to a concentration of 7·5 μg cortisol/ml culture medium but that regressive changes were beginning after approximately 5–9 days of contact with the cortisol. By 12 days of exposure to this concentration of cortisol, Singh and Hardy (1975) reported extensive disorganization of follicular structure in the skin explants.

The ratio of the concentrations of cortisol required to inhibit hair follicles in cultured skin, 7·5 μg/ml medium (Singh and Hardy 1975), and of flumethasone required to cause wool fibre shedding here, about 7 ng/ml plasma, is approximately 1000:1, a proportion not markedly dissimilar to 700:1 reported for the same two chemicals in the liver glycogen deposition assay (Fried 1960) or from 730:1 in the granuloma inhibition assay in rats (Boland 1961).

The experiments reported here are the first, as far as we are aware, to report flumethasone concentrations in the circulation of sheep. As a generalization, approximately one-sixth to one-seventh of the quantity of dexamethasone previously shown to cause complete fleece shedding (Panaretto and Wallace 1978) was required as flumethasone. The question as to whether flumethasone, its metabolites, or both, inhibited wool growth in the experiments must remain open.

Plasma flumethasone concentrations during an 8-day infusion showed one important feature, namely the tendency to maintain stable plasma concentrations throughout the last 4 days of the infusion period following the initial peak seen during the first 24–48 h of infusion. This contrasted with the plasma dexamethasone concentration patterns following the infusion of dexamethasone (Panaretto and Wallace 1978). The infusion of flumethasone over relatively short periods in experiment 3 tended to give flumethasone concentration profiles similar to those seen during the infusion of dexamethasone over 8 days. It is possible that the relatively stable flumethasone concentrations seen here during the last 4 days of an 8-day infusion were due, in part, to the slow hydroxylation of the molecule at its 6 position. Betamethasone and dexamethasone, which differ from flumethasone mainly in lacking a fluorine atom at position 6 of the molecule, have been shown to be transformed by 6 β-hydroxylation in human beings and rats (Butler and Gray 1970; Rice *et al.* 1974). This process is relatively slow when compared with metabolism of ring A in the cortisol molecule to the tetrahydro compounds and cortol. The relatively very slow clearance

rates for flumethasone during the first 48 h of infusion offered similar options to those presented by the metabolism of dexamethasone in that flumethasone might be initially inhibiting its own enzymic metabolism (Parke 1976). There were, however, no increases in flumethasone concentrations during the last part of the infusions as seen during dexamethasone infusions (Panaretto and Wallace 1978).

The glucocorticoid analogues have three possible ways in which their potency is enhanced over cortisol—their metabolism is slower, their binding to plasma proteins is less, and they may exhibit greater affinity for binding sites. The first of these attributes was supported by the MCR for flumethasone which appeared to be, in the majority of cases here, about 200–700 ml/min, a value one-quarter to three-quarters of that of cortisol in undisturbed sheep (Panaretto and Vickery 1972).

When it occurred, fleece shedding followed a reproducible pattern. Generally if any wool was shed then this was from the belly and ventral trunk regions. The next most frequently shed wool lay in the lumbar, sacral and lateral thigh regions. The wool on the dorsal thoracic region was less likely to be shed and the probability of complete shedding decreased even further in the cervical and cephalic regions. These last regions were most likely to contain substantial proportions of continuous fibres and hence cause the retention of the wool covering the area. In those instances where the fleece was retained as a whole, wool in the anterior regions frequently showed few or no shed fibres while the proportion of shed fibres increased in the postero-ventral direction. The importance of continuous fibres preventing the spontaneous shedding of 'broken' fleeces has been emphasized by Ryder (1971).

It is of interest that the anterior or first-formed follicle groups (Carter and Hardy 1947) and the relatively denser dorsal follicle groups (Young and Chapman 1958) were most resistant to the effects of flumethasone. It is a matter of speculation whether the treatment was reversing the original order of follicle group development, and although the possibility exists that the greater density of fibres along the dorsum, relative to the somewhat sparser follicle populations ventrally and cervically, may reduce the quantity of hormone available to each fibre target, this explanation appears to be remote when the response of cervical follicles is taken into account. Another related question which needs additional research is whether the unbroken fibres represented a random sample of the fibre population in any region.

The physiological causes for anagen follicles proceeding to telogen ones are unknown. Several features in sheep here resembled to some extent, by chance or otherwise, the appearance of wool during natural shedding in sheep. These included a proportion of continuous fibres among those shed, so preventing casting of the fleece (see Ryder 1971), and ventro-dorsal shedding (Slee 1959). Furthermore the formation of 'brush ends' in fibres has been described following steroid treatment (Chapman and Bassett 1970; Chapman and Panaretto, unpublished data) and in natural shedding (Ryder 1974). The differential responses of wool follicles to exogenous glucocorticoids both between animals and over different body regions of any individual could imply differences in sensitivity between follicular cells. This suggestion is not completely novel; for example, Kaiser and Edelman (1977) discussed differences in sensitivity to glucocorticoids between large and small thymic lymphocytes in rats. The possibility exists that a similar situation with respect to wool follicles occurs here. Finally, a proportion of continuous wool fibres large enough to retain the shed fibres on the sheep, but capable of being disrupted at the will of the wool harvester

when sufficient regrowth has occurred, might provide an answer to an important problem in wool harvesting by the use of chemicals. This is casting of the fleece before regrowth has occurred, which loses much wool and leaves a *nude* sheep (e.g. Roberts and McMahon 1972). Many factors that will influence this suggestion, including the regional distribution of shed fibres, are being investigated here.

The quantity of intravenously administered flumethasone required to cause complete shedding of the fleece in sheep here ($1 \cdot 2$–$1 \cdot 4$ mg/kg$^{0 \cdot 75}$) exceeded by a factor of about $2 \cdot 5$ the amount ($2 \cdot 5$ mg daily for 3 days) shown to induce parturition in pregnant ewes before the 130th day of gestation (Emady *et al.* 1974). This quantity of flumethasone did not differ greatly from that ($0 \cdot 66$ mg/kg$^{0 \cdot 75}$) which causes shedding of wool fibres in many of the sheep in our experiments. Thus it is probable that the doses of flumethasone used here would cause the onset of premature parturition in pregnant ewes.

Flumethasone-treated animals required approximately 40–60 days to re-establish their wool growth to its pretreatment levels. This interval was longer than that (approximately 30 days) reported by Panaretto and Wallace (1978) for recovery in dexamethasone-treated sheep. This difference may in part have been due to the dietary increase from 600 to 1000 g food daily given to the dexamethasone-treated sheep after infusion.

The suppression of endogenous cortisol secretion during flumethasone treatment was similar to that seen during dexamethasone infusion (Panaretto and Wallace 1978). Recovery of endogenous cortisol secretion was rapidly established and generally coincided with the fall in plasma flumethasone concentration.

There was a marked tendency for flumethasone treatment to result in sheep temporarily refusing to eat the whole of their food ration. This tendency was unmistakable after intravenous infusion of flumethasone in experiments 1 and 2 and very much exacerbated in experiment 3 when the intravenous infusion rate of flumethasone was increased. These effects, however, were transitory and this was shown by sheep in experiment 2 which showed some tendency to leave some of their food either during or after infusion. However, each animal recovered and readily ate its ration when the amount was increased to 1000 g daily 14 days after infusion finished. Feed refusals, resulting from elevated glucocorticoids in sheep, have been reported by Doney and Smith (1969). It is speculatively suggested that feed refusals here were due to metabolic disorders due to the effects of the steroid hormone. The possibility that flumethasone affects organs such as the liver may need to be considered here (Wiener *et al.* 1968; Thompson *et al.* 1971). The quantities of feed left and the frequency of leaving food were not significant in either of the experiments where subcutaneous or intraruminal routes were used.

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