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Defleecing Effect of Betamethasone and other Long-acting Corticosteroids, their Influence on Wool Growth and some Physiological Processes in Sheep

B. J. McDonald, G. Suter and P. S. Hopkins

Queensland Department of Primary Industries,
Animal Research Institute, Yeerongpilly, Qld 4105.

Abstract

The defleecing effects of the long-acting derivatives of prednisolone, triamcinolone and dexamethasone were compared with those of betamethasone alcohol when these steroids were administered at the rate of 3·3 mg/kg liveweight in three equal intramuscular injections of 1·1 mg to Merino wethers. Prednisolone showed no defleecing activity whereas the other steroids produced positive but variable responses. Prolonged depression of wool growth was evident following treatment with dexamethasone esters.

Betamethasone alcohol injected intramuscularly at 1·1 mg/kg daily for 3 days produced a similar defleecing response to intravenous infusion of 3·3 mg/kg betamethasone phosphate over 8 days. A range of dose rates (0·3–3·3 mg/kg) of betamethasone as multiple and single intramuscular injections indicated that the minimum effective defleecing dose was approximately 2·1 mg/kg.

The response to simultaneous administration of betamethasone and insulin or chlorpropamide (to increase glucose utilization) and glucose or xylazine (to increase hyperglycaemia) suggested that the gluconeogenic role of this steroid had little effect on fibre shedding. Thyroxine (300 μg per sheep) administered on the first day with an injection of betamethasone (0·9 mg/kg), and alone daily for 20 days thereafter, did not influence the changes in wool production resulting from betamethasone treatment.

These results are discussed in relation to the molecular structure and physiological characteristics of a potentially specific defleecing steroid.

Introduction

Extensive studies of the use of the synthetic corticosteroids dexamethasone and flumethasone as defleecing agents in sheep have been reported by Panaretto *et al.* (1975), Panaretto and Wallace (1978*a*, 1978*b*), Leish and Panaretto (1978) and Panaretto (1979). These papers have described wool growth responses, fibre shedding and fleece casting and the production of some endogenous hormones and plasma steroid profiles induced by intraperitoneal (i.p.), intravenous (i.v.), and subcutaneous (s.c.) injections, oral dosing and i.v. infusion of various forms of the two steroids. Gordon (1980) reported the effect of intramuscular (i.m.) and s.c. injection of dexamethasone isonicotinate on the tensile strength of wool. Reis and Panaretto (1979) in a review of much of this work noted the considerable variation in the extent of fibre shedding both between animals and between regions of the body.

The experiments reported in this paper were designed to expand on the observations of previous workers in this field by making comparisons of the defleecing effectiveness of long-acting derivatives of prednisolone, triamcinolone, dexamethasone and betamethasone. Intramuscular injection of betamethasone alcohol was compared with i.v. infusion of betamethasone phosphate. A range of dose rates of betamethasone as multiple and single i.m. injections was evaluated with the aim of establishing a single-dose method of administration. The gluconeogenic effect of betamethasone and its tendency to elicit hypothyroidism were also evaluated in an attempt to understand something of the mechanism of defleecing activity.

Materials and Methods

Sheep

In all, 163 Merino wethers 2–3 years old and in full wool were individually housed in cages. Each animal was offered 800 g lucerne pellets daily and feed refusals were recorded.

Steroid Analogues

Injections

The six steroids administered by i.m. injection in the posterior thigh muscles (M. semitendinosus, M. semimembranosus) were:

(1) prednisolone 21-trimethylacetate [11β,17α-dihydroxy-21-pivaloyloxypregna-1,4-diene-3,20-dione] as Vecortenol (Ciba Company Pty Ltd, Lane Cove, N.S.W.);
(2) triamcinolone 16,17-acetonide [9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxy-pregna-1,4-diene-3,20-dione] as Vetalog (E. R. Squibb & Sons Pty Ltd, Noble Park, Vic.);
(3) dexamethasone 21-isonicotinate [9α-fluoro-11β,17α-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione-21-(4-pyridine-carboxylate)] as Voren Depot (Boehringer Ingelheim Pty Ltd, Artarmon, N.S.W.);
(4) dexamethasone 21-trimethylacetate [9α-fluoro-11β,17α-dihydroxy-16α-methyl-21-pivaloyl-oxypregna-1,4-diene-3,20-dione] as Opticortenol (Ciba-Geigy Australia Ltd, Lane Cove, N.S.W.);
(5) betamethasone alcohol [9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione] as Betsolan Injection, 2 mg/ml (Glaxo Australia Pty Ltd, Boronia, Vic.);
(6) betamethasone alcohol as Betsopart, 10 mg/ml (Glaxo Laboratories New Zealand Ltd, Palmerston North, New Zealand).

Infusions

Betamethasone disodium phosphate [9α-fluoro-11β,17α-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 21-disodium phosphate] as Betsolan Soluble Injection (Glaxo Australia Pty Ltd, Boronia, Vic.) was extended in sterile 0·9% (w/v) sodium chloride solution to provide the equivalent of 0·413 mg betamethasone alcohol per 50 ml of the final solution which was infused at a constant rate over 24 h using a Watson-Marlow MHRE/7/Delta infusion pump. Cardiac-directed vinyl catheters of i.d. 0·63 mm, o.d. 1·40 mm [The Boots Company (Australia) Pty Ltd] were implanted by jugular venipuncture.

Concomitant Treatments

Injections

The following compounds were administered by s.c. injection in the inguinal or axillary regions: biphasic insulin as Rapitard 40 units/ml and Rapitard 80 units/ml (Novo Laboratories Pty Ltd, North Ryde, N.S.W.); chlorpropamide as Diabenese 250 mg tablets (Pfizer Pty Ltd, West Ryde, N.S.W.) suspended in 5 ml sterile 0·9% (w/v) sodium chloride per tablet; xylazine as Rompun 20 mg/ml (Bayer Australia Ltd, Botany, N.S.W.); L-thyroxine, sodium salt (Calbiochem, San Diego, U.S.A.) dissolved in sterile 0·9% (w/v) sodium chloride to provide 300 μg per 5 ml of the final solution.

Infusions

D-Glucose and sodium chloride (both A.R. grade, Ajax Chemicals) were dissolved in distilled water to provide an infusate containing 30% (w/v) D-glucose and 0·9% (w/v) sodium chloride. A Desaga 131900 peristaltic pump was used to deliver the infusate at the rate of 5·5 g D-glucose per hour.

Measurements

Liveweight

Experimental animals were weighed every 7 days except during infusion in experiment 2.

Wool Growth

Wool on the left midside of each sheep was dye-banded at skin level with a solution of Durafur Black R (I.C.I. Australia Ltd) as described by Wheeler *et al.* (1977). Dye bands were applied 42 days prior to, and at the commencement of, steroid treatment. Pretreatment linear wool growth (millimetres per week) was calculated as the mean of three measurements taken at 7-day intervals within the 21 days immediately prior to treatment. Sheep were allocated to experimental groups by stratified randomization according to their pretreatment linear wool growth values. Linear wool growth measurements were then made at 7- or 14-day intervals following treatment. Measurements ceased when wool was cast at the dye-banded site or when the experiment was terminated. Linear wool growth measurements were chosen since they provided a reasonably sensitive indicator of changes that occurred over short periods of time. The resulting dye lines on both shedding and non-shedding sheep were always straight and discrete thereby affording a reliable objective monitor of changes induced.

In experiments 4, 5 and 10, additional wool samples were clipped (at 7- or 14-day intervals) at skin level on a measured patch on the right midside of each sheep to determine wool production. The greasy wool clippings were cleaned by repeated washings with light petroleum (Shell X4) and any non-woollen or extraneous particles were removed from the air-dry sample. The clean wool was then dried at 105°C to constant weight in a ventilated oven and the oven-dried weight obtained.

Fibre shedding and fleece casting

The following shedding categories were used to evaluat subjectively the effect of treatment one wool fibre shedding and fleece casting: 1, most fibres lay *outside the follicle* and the fleece was spontaneously cast; 2, a proportion of fibres lay outside the follicle and the balance remained continuous and there was regional fleece casting; 3, a proportion of fibres lay outside the follicle and the balance remained continuous and there was no fleece casting; 4, a proportion of fibres lay outside the follicle on the belly, legs and flank *only* and the balance remained continuous; and 5, there was no discontinuity of wool fibres. A minimum effective dose was ascribed to fibre-shedding qualities delineated in categories 1, 2 and 3 assessed in all sheep in a group up to and including 63 days following treatment.

Experimental Procedures

Details of treatments (steroids, duration of dosing, dose rates and methods of administration) in experiments 1–5 are shown in Table 1. Steroid dose rates are expressed as rates for the free alcohol.

Experiment 1

The defleecing effectiveness of long-acting derivatives of prednisolone, triamcinolone and dexamethasone was compared with that of betamethasone alcohol as an aqueous microcrystalline suspension. The long-acting compounds would have been slowly absorbed from administration sites due to modification of their molecular structure by esterification of the hydroxyl group at C21 or by acetonide formation across C16 and C17. The treatments provided comparisons of the composite effect of steroid activity and rate of absorption.

Experiment 2

Equal doses (3.3 mg/kg) of soluble betamethasone disodium phosphate and microcrystalline betamethasone alcohol were infused intravenously and injected i.m. respectively to compare the defleecing activity of betamethasone using these two routes of administration for different durations.

Experiment 3

The defleecing performance of a range of dose rates of betamethasone alcohol (formulated as 2 mg/ml) was evaluated. Low dose rates of betamethasone were used in this experiment to establish a minimum effective defleecing dose (MEDD) of this steroid under the dosing regimen described in Table 1.

Table 1. Steroid treatments, dose rates and regimens for sheep in experiments 1–5

Expt No.	Treatment	Total cortico-steroid dose (mg/kg)	Duration of treatment (days)	No. of sheep	Mean sheep liveweight ± s.e.m. (kg)
1[A]	Control			4	29·4±1·4
	Prednisolone trimethylacetate	3·3	3	4	30·8±1·3
	Triamcinolone acetonide	3·3	3	4	28·5±0·8
	Dexamethasone isonicotinate	3·3	3	4	29·9±2·0
	Dexamethasone trimethylacetate	3·3	3	4	28·5±0·5
	Betamethasone	3·3	3	4	27·9±2·1
2	0·9% NaCl[B]		8	3	30·0±2·5
	Betamethasone[A]	3·3	3	3	30·3±2·0
	Betamethsone-21-phosphate[B]	3·3	8	3	30·5±1·6
3[A]	Control			3	44·2±2·7
	Betamethasone	2·1	3	3	44·7±2·2
	Betamethasone	1·5	3	3	46·0±0·8
	Betamethasone	0·9	3	3	48·2±1·9
	Betamethasone	0·3	3	3	44·7±2·9
4[C]	Control			4	50·9±2·8
	Betamethasone	3·3	1	4	46·7±3·0
	Betamethasone	2·1	1	4	48·3±2·3
	Betamethasone	1·5	1	4	48·4±1·2
	Betamethasone	0·9	1	4	45·6±1·0
5[C]	Control			5	45·8±2·6
	Betamethasone	0·9	1	5	48·8±1·8
	Betamethasone	0·3	1	5	47·6±2·4

[A] Injected i.m. as three equally divided doses each administered daily.
[B] Administered by i.v. infusion.
[C] Administered by single i.m. injection.

Experiments 4 and 5

Betamethasone alcohol (10 mg/ml) was used in these experiments to establish a MEDD of betamethasone administered by single i.m. injection.

Experiments 6–9

These experiments were designed to study the extent to which the gluconeogenic effect of betamethasone influenced either the defleecing effect or the hypoactive linear wool growth response observed in previous experiments. The compounds (Table 2) administered concomitantly with betamethasone were used to increase glucose utilization (insulin and chlorpropamide) or increase hyperglycaemia (glucose and xylazine).

Experiment 10

The effect of exogenous thyroxine on the hypoactive wool growth and shedding responses to betamethasone treatment were evaluated in this experiment. Thyroxine was administered simultaneously with betamethasone and for 20 days thereafter (Table 2).

Statistical Analyses

Wool growth data were analysed by analysis of variance. Where appropriate, the data were adjusted by covariance for differences in the pretreatment wool growth. When wool was cast at the dye-banded site wool growth measurements were estimated by the analysis.

Table 2. Experimental treatments, betamethasone dose rates and regimens for sheep in experiments 6–10

Betamethasone provided as three equally divided doses each administered i.m. daily in experiments 6–9 and as one dose in experiment 10. Three sheep used in all experiments except experiment 9 in which four sheep were used

Expt No.	Total dose of beta-methasone (mg/kg)	Duration of dose (days)	Concomitant treatment				Mean sheep liveweight ±s.e.m. (kg)
			Compound	Daily dose per sheep	Duration (days)	Day of treat-ment	
6							48·0±2·5
	2·1	3					39·7±1·7
			Glucose[A]	132 g	6	1–6	48·0±1·0
	2·1	3	Glucose[A]	132 g	6	1–6	42·3±1·6
7	3·3	3					53·0±1·8
			Insulin	64 units	3	1–3	52·2±1·1
	3·3	3	Insulin	64 units	3	1–3	50·3±2·4
8							42·8±2·2
			Insulin	120 units	2	1–2	43·7±0·9
	2·1	3					44·2±1·6
	3·3	3					42·8±1·2
	2·1	3	Insulin	120 units	2	1–2	41·5±2·0
	3·3	3	Insulin	120 units[B]	3	1–3	43·7±2·5
9							27·2±1·7
	2·1	3					28·3±0·9
	3·3	3					28·4±2·7
			Chlorpropamide	250 mg	4	0–3	26·9±1·3
			Xylazine	1·2 mg/kg[C]	3	1–3	29·9±0·8
	2·1	3	Chlorpropamide	250 mg	4	0–3	27·6±0·6
	3·3	3	Chlorpropamide	250 mg	4	0–3	27·8±1·5
	2·1	3	Xylazine	1·2 mg/kg[C]	3	1–3	25·4±2·6
10	0·9	1					39·3±1·5
			Thyroxine	300 μg	21	1–21	37·3±1·6
	0·9	1	Thyroxine	300 μg	21	1–21	43·3±0·6

[A] Administered by i.v. infusion, other concomitant treatments administered by s.c. injection.
[B] Dose reduced to 64 units on the third day.
[C] Administered as 0·4 mg/kg at 0830 h and 0·8 mg/kg at 1630 h.

Results

Liveweight and Feed Refusals

Steroid administration produced no evidence of loss in liveweight or prolonged feed refusal. Occasionally a sheep treated with betamethasone consumed only part of the daily ration; however, the residue and the ration offered on the following day were generally eaten by the end of that day.

Experiment 1: *Injection of Long-acting Steroid* (*see Figs 1a and 1b*)

The mean linear wool growth of sheep treated with prednisolone trimethylacetate was similar to that of untreated control sheep. Prednisolone treatment did not produce fibre shedding (Table 3). The administration of dexamethasone isonicotinate and trimethylacetate depressed linear wool growth for at least 28 days (days 21–49) and at least 35 days (days 14–49) respectively whereas with betamethasone treatment maximum depression of linear wool growth occurred at 14 days and recovery was complete at 28 days. Sheep treated with betamethasone showed significantly greater

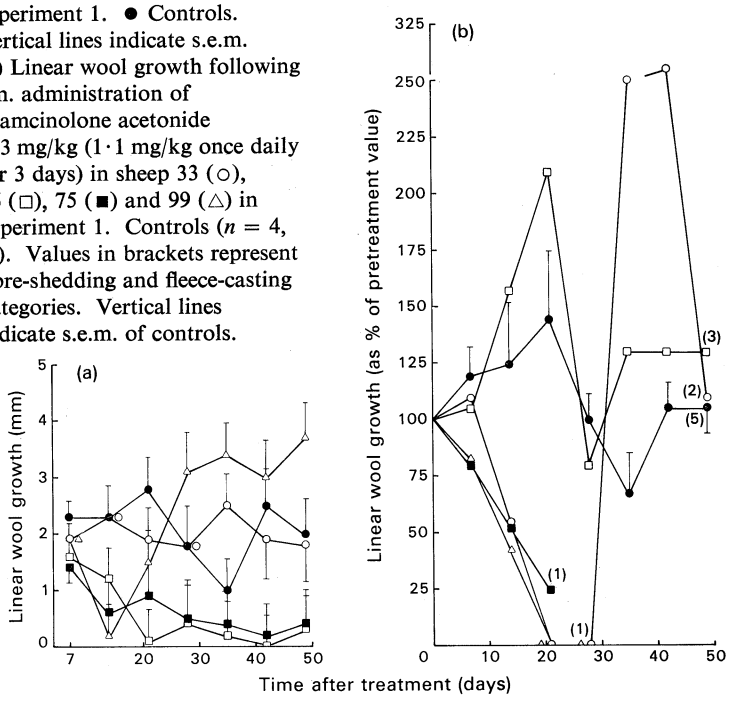

Fig. 1. (*a*) Adjusted mean linear wool growth of groups of sheep ($n = 4$) following i.m. administration of 3·3 mg/kg prednisolone trimethylacetate (○), dexamethasone isonicotinate (□), dexamethasone trimethylacetate (■) and betamethasone (△) in experiment 1. ● Controls. Vertical lines indicate s.e.m. (*b*) Linear wool growth following i.m. administration of triamcinolone acetonide 3·3 mg/kg (1·1 mg/kg once daily for 3 days) in sheep 33 (○), 46 (□), 75 (■) and 99 (△) in experiment 1. Controls ($n = 4$, ●). Values in brackets represent fibre-shedding and fleece-casting categories. Vertical lines indicate s.e.m. of controls.

linear wool growth than those treated with dexamethasone isonicotinate and trimethylacetate at days 35 and 42 ($P < 0{\cdot}01$ and $P < 0{\cdot}05$) respectively. At 49 days after treatment both dexamethasone trimethylacetate and betamethasone caused complete fleece casting in one sheep, regional casting in two and fibre shedding with no fleece casting in the other (Table 3). Dexamethasone isonicotinate induced complete fleece casting in one sheep and fibre shedding in the other three. When wool was cast by or plucked from sheep in the two dexamethasone-treated groups at day 49 there was little wool cover remaining on the skin, indicating that minimal wool regrowth had occurred. In contrast, sheep treated with betamethasone were covered with dense wool regrowth following plucking or casting.

The individual linear wool growth responses of sheep treated with triamcinolone acetonide are presented in Fig. 1*b*. Three sheep (33, 75 and 99) exhibited maximum

depression of linear wool growth at day 21. Sheep 75 and 99 were casting their fleeces by days 28 and 35 respectively and were easily defleeced by hand on day 42 leaving a short dense stubble on the skin. Sheep 33 underwent a period of hyperactive linear wool growth between days 28 and 42 (255% of pretreatment value) and returned to pretreatment levels at day 49 after showing regional fleece casting at day 42. The linear wool growth of sheep 46 showed hyperactivity to day 21 when it represented 210% of pretreatment value, resulting in fibre shedding without fleece casting at day 42.

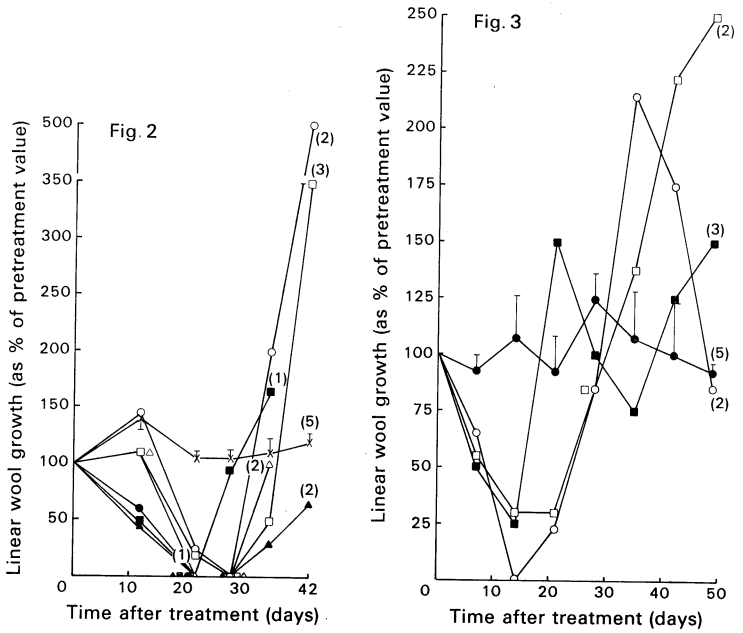

Fig. 2. Linear wool growth after i.v. infusion of 3·3 mg/kg betamethasone as 21-phosphate (sheep 65, ○; 68, □; and 71, △) or i.m. injection of 3·3 mg/kg betamethasone alcohol (sheep 03, ●; 67, ■; and 63, ▲) in experiment 2. Controls (n = 3, ×). Values in brackets represent fibre-shedding and fleece-casting categories. Vertical lines indicate s.e.m. of controls.

Fig. 3. Linear wool growth following i.m. administration of betamethasone 2·1 mg/kg (0·7 mg/kg once daily for 3 days) in sheep 57 (○), 49 (□) and 76 (■) in experiment 3. Controls (n = 3, ●). Values in brackets represent fibre-shedding and fleece-casting categories. Vertical lines indicate s.e.m. of controls.

Experiment 2: Intramuscular Injection and Intravenous Infusion of Betamethasone

The administration of betamethasone in three equal doses i.m. over 3 days caused maximum depression of linear wool growth in all sheep at day 21 while the same total dose of betamethasone administered by i.v. infusion over 8 days resulted in maximum depression of linear wool growth in one sheep (71) at day 21 and in two sheep (65 and 68) at day 28 (Fig. 2). Hyperactive linear wool growth was observed in two sheep (65 and 68) from the infused group. Only one sheep (67) from the injected group had exceeded control linear wool growth by day 42; however, no values were recorded from sheep 03 owing to casting of wool on the measurement site after day 21. The injection dosing regimen induced complete fleece casting in two sheep (03 and 67) while infusion did not elicit this response.

Experiment 3: *Multiple Injections Minimum Effective Defleecing Dose of Betamethasone*

When betamethasone was administered at 2·1, 1·5, 0·9 and 0·3 mg/kg, only 2·1 mg/kg produced a reduction in linear wool growth of 70% or more. Changes in linear wool growth resulting from this dose rate are shown in Fig. 3. When compared with pretreatment values, minimum linear wool growth occurred at day 14 for the three sheep in this group. Sheep 57 grew no wool between days 7 and 14 while the linear wool growth of 49 and 76 was reduced to 30 and 25% of their pretreatment values respectively. At day 35 sheep 76 had returned to linear wool growth about control values whereas 57 and 49 underwent a hyperactive phase of linear wool growth. At day 49 two sheep showed evidence of regional fleece casting and the other fibre shedding. Two sheep dosed with 1·5 mg/kg and two dosed with 0·9 mg/kg betamethasone showed evidence of fibre shedding on the belly, legs and flank with the balance of fibres remaining continuous.

Experiments 4 and 5: *Single Injection Minimum Effective Defleecing Dose of Betamethasone*

Changes in clean dry wool production followed the i.m. administration of single doses of betamethasone alcohol (10 mg/ml) at the rate of 3·3, 2·1, 1·5, 0·9 and 0·3 mg/kg. Betamethasone at 3·3 mg/kg reduced wool production to 12·6, 1·4 and 19·5% of the pretreatment value at days 21, 35 and 49 respectively. Wool growth of the groups receiving 2·1, 1·5, 0·9 and 0·3 mg/kg betamethasone showed a similar pattern of changes; however, depression in wool growth was less severe than that elicited by 3·3 mg/kg. The clean wool production of sheep injected with betamethasone at 0·3 mg/kg had returned to that of control sheep at day 49, whereas none of the other dose rates consistently produced this response. The slow regrowth elicited by this steroid at 3·3 mg/kg was reflected in the shedding assessment at day 49 when no response was evident (Table 3). All of the sheep in this group had begun to cast their fleece by the 63rd day after treatment and were scored as category 1. Betamethasone at 2·1 mg/kg caused two sheep to cast their fleece and two to shed fibres without fleece casting. At both 1·5 and 0·9 mg/kg one sheep cast most of its fleece and one cast wool from a particular region. One sheep at the 0·9 mg/kg dose shed fibres but did not cast any fleece. Most fibres remained continuous on the head and neck of all sheep that received 1·5 and 0·9 mg/kg betamethasone and could not be removed by hand even though in some cases wool was spontaneously cast from other regions.

Assessment of fibre shedding and fleece casting (Table 3) 49 days after treatment with betamethasone in experiment 5 showed that one of five sheep did not respond to 0·9 mg/kg and three of five sheep injected with 0·3 mg/kg showed no response. Wool on the head and neck of sheep that did respond to treatment could not be removed by hand as most fibres in this region remained continuous.

Experiment 6: *Glucose Infusion and Betamethasone Injection*

Changes in linear wool growth following i.m. injection of betamethasone 2·1 mg/kg, i.v. infusion of glucose 5·5 g/h for 6 days and betamethasone injection with glucose infusion are shown in Fig. 4. Treatment with betamethasone, glucose and betamethasone plus glucose depressed linear wool growth at day 14 when all three treatments elicited significantly lower wool growth than the controls ($P < 0·01$). Wool

growth resulting from treatment with betamethasone plus glucose was less than that from betamethasone alone which was less than that from glucose alone ($P < 0{\cdot}01$). When compared with controls the individual effects of betamethasone and glucose show an additive effect on linear wool growth in the combination treatment. Both

Table 3. Effects of treatments on fibre shedding and fleece casting for experiments 1, 4, 5, 6, 9 and 10
Categories 1–5 for fibre shedding and fleece casting assessment as defined in Materials and Methods

Expt No.	No. of sheep	Treatment	Steroid dose rate (mg/kg)	Day 42: No. of sheep in categories: 1	2	3	4	5	Day 49: No. of sheep in categories: 1	2	3	4	5
1	4	Control						4					4
	4	Prednisolone	3·3					4					4
	4	Triamcinolone	3·3	2	1	1			2	1	1		
	4	Dexamethasone isonicotinate	3·3	1				3	1		3		
	4	Dexamethasone trimethylacetate	3·3			1		3	1	2	1		
	4	Betamethasone	3·3	1	1	1	1		1	2	1		
4	4	Control						4					4
	4	Betamethasone	3·3					4					4
	4	Betamethasone	2·1	1	1			2	2		2		
	4	Betamethasone	1·5	1	1			2	1	1			2
	4	Betamethasone	0·9	1	1	1		1	1	1	1		1
5	5	Control						5					5
	5	Betamethasone	0·9		1	2		2		3	1		1
	5	Betamethasone	0·3		1	1		3		1	1		3
6	3	Control						3					
	3	Betamethasone	2·1		1	2							
	3	Glucose						3					
	3	Betamethasone + glucose	2·1		2	1							
9	4	Control						4					4
	4	Betamethasone	2·1		1	3				1	3		
	4	Betamethasone	3·3		1	3			1	1	2		
	4	Chlorpropamide						4					4
	4	Xylazine						4					4
	4	Betamethasone + chlorpropamide	2·1			1		3		1	2		1
	4	Betamethasone + chlorpropamide	3·3		1	3				1	3		
	4	Betamethasone + xylazine	2·1	1		3			1	1	2		
10	3	Betamethasone	0·9	1	1			1	1	1			1
	3	Thyroxine						3					3
	3	Betamethasone + thyroxine	0·9			2		1			2		1

treatments involving betamethasone underwent a hyperactive phase of linear wool growth of similar magnitude and returned to near the control value on day 42. The greater reduction in linear wool growth caused by betamethasone plus glucose did not produce enhanced fibre shedding or fleece casting when compared to betamethasone alone (Table 3).

Experiments 7 and 8: Insulin Injection and Betamethasone Injection

Treatment with 120 or 64 units biphasic insulin for 2–3 days during administration of betamethasone at the rate of 3·3 or 2·1 mg/kg (Table 2) did not appear to produce changes in linear wool growth or fibre shedding and fleece casting results differing from those elicited by the two dose rates of betamethasone alone. The death of two sheep in the group receiving 120 units insulin alone and in the group receiving 120 units insulin with 2·1 mg/kg betamethasone indicates that acute hyperinsulinaemia was produced; however, the absence of symptoms of hyperinsulinaemia in the group receiving 3·3 mg/kg betamethasone with insulin illustrates the increased resistance to insulin resulting from the higher dose rate of steroid.

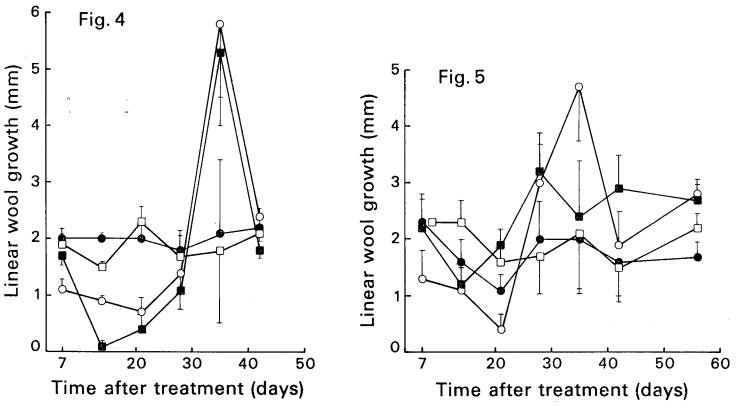

Fig. 4. Adjusted mean linear wool growth of groups of sheep ($n = 3$) following administration of betamethasone (2·1 mg/kg i.m., ○), glucose (792 g/sheep i.v., □) or a combination of each (■) in experiment 6. ● Controls. Vertical lines indicate s.e.m.

Fig. 5. Adjusted mean linear wool growth of groups of sheep ($n = 4$) following administration of betamethasone (2·1 mg/kg i.m., ○), chlorpropamide (1 g/sheep s.c., □) or a combination of each (■) in experiment 9. ● Controls. Vertical lines indicate s.e.m.

Experiment 9: Chlorpropamide and Xylazine Injection and Betamethasone Injection

Concurrent treatment with 250 mg chlorpropamide daily with 3·3 mg/kg betamethasone or 1·2 mg/kg xylazine daily with 2·1 mg/kg betamethasone (Table 2) did not alter the linear wool growth or fibre shedding and fleece casting response to treatment with 3·3 or 2·1 mg/kg betamethasone alone. The changes in linear wool growth following treatment with betamethasone 2·1 mg/kg, chlorpropamide 250 mg daily and betamethasone plus chlorpropamide and those of controls are shown in Fig. 5. The result of treatments must be tempered by the fact that linear wool growth (mean ± s.e.m.) of control animals fell from 2·30 ± 0·5 mm on day 7 to 1·6 ± 0·4 mm on day 14 and 1·1 ± 0·3 mm on day 21. However, it is assumed that this change was representative of all animals in this experiment. Betamethasone at 2·1 mg/kg produced maximum depression in linear wool growth to 0·4 ± 0·3 mm at day 21. Where chlorpropamide was administered with 2·1 mg/kg betamethasone maximum depression in linear wool growth to 1·2 ± 0·4 mm occurred at day 14. At day 21 betamethasone plus chlorpropamide treatment resulted in significantly higher linear wool growth than treatment with betamethasone alone ($P < 0·01$). This suggests that

250 mg chlorpropamide daily prevented the major depression in linear wool growth that occurred following treatment with betamethasone at 2·1 mg/kg but not at 3·3 mg/kg, this dose producing depression in linear wool growth to 0·5±0·4 and 0·2±0·3 mm at days 14 and 21 respectively. Table 3 shows that fibre shedding and fleece casting in three of the four animals treated with betamethasone plus chlorpropamide was similar to that of the animals receiving betamethasone alone. Although the linear wool growth of the group treated with 2·1 mg/kg betamethasone and chlorpropamide was similar to that of controls at day 14 and exceeded that of controls at days 21 and 28, three of the four sheep in the treated group showed evidence of fibre shedding, whereas fibre shedding did not occur in animals in the untreated control group. Chlorpropamide also appeared to delay the onset of fibre shedding and fleece casting resulting from treatment with betamethasone because the maximum response from concomitant treatment with these compounds was not evident until day 49 whereas the maximum response to betamethasone treatment alone was seen at day 42. Chlorpropamide also appeared to inhibit the response to betamethasone in the case of one animal that did not shed many fibres following treatment.

Experiment 10: *Thyroxine Injection and Betamethasone Injection*

A single i.m. dose of betamethasone 0·9 mg/kg, s.c. injections of thyroxine 300 μg per sheep for 21 days and the administration of a combination of these treatments changed the daily mean (±s.e.m.) clean wool production of groups of sheep to 0·29±0·16, 0·77±0·16 and 0·35±0·16 mg/cm^2, 0·58±0·15, 0·77±0·15 and 0·54±0·15 mg/cm^2 and 0·82±0·08, 0·79±0·08 and 0·64±0·08 mg/cm^2 respectively at days 21, 35 and 49. The fibre-shedding and fleece-casting assessment (Table 3) shows similar results to those previously observed following treatment with 0·9 mg/kg betamethasone. There was some indication that simultaneous treatment with thyroxine may have reduced the response to betamethasone; however, a greater number of animals in the treatment groups would be necessary to confirm this effect.

Discussion

The steroids evaluated in these experiments have a common basic molecular structure which is a pregnane nucleus consisting of four rings with 21 carbon atoms. A double bond between C4 and C5 and a ketone group at C3 are necessary for corticosteroid activity while fluorination in the 9α position enhances all biological activities of the corticosteroids (Sayers and Travis 1970). Esterification of the hydroxyl group at C21 provides long duration of action by reducing the rate of absorption from the injection site and is unlikely to influence the fundamental defleecing activity of the compound as the 21-hydroxyl group is not necessary for most biological activities (Sayers and Travis 1970). Acetonide formation across C16 and C17 in the case of triamcinolone also reduces the rate of absorption. The influence of the acetonide group on defleecing activity is discussed further in this paper.

Small differences between the molecular structures of the long-acting derivatives of prednisolone, triamcinolone, dexamethasone and betamethasone appeared to result in major differences in their activity as defleecing agents. Prednisolone trimethylacetate produced no evidence of formation of fibre brush ends. Triamcinolone, dexamethasone and betamethasone each have a α-fluorine atom at C9. However, they differ by also having a α-hydroxyl, α-methyl and a β-methyl group at C16

respectively. Dexamethasone isonicotinate and trimethylacetate and betamethasone showed basic defleecing activity indicating that the presence of a fluorine atom at C9 and a radical at C16 are necessary for defleecing activity when the corticosteroid is provided as three divided doses each administered daily as one i.m. injection. Chapman and Bassett (1970) showed that injection of cortisol (which has no substituents on C9 or C16) produced shedding of wool fibres. However, they administered daily doses during four successive periods, each of 21 days duration, thus removing the requirements for reduced rate of absorption and metabolism. The insoluble esters of dexamethasone appeared to produce prolonged depression in linear wool growth compared with betamethasone alcohol where sheep had exceeded their normal linear wool growth 28 days after treatment. The prolonged depression in linear wool growth caused by the dexamethasone esters resulted from either the esterification of the hydroxyl group at C21 causing prolonged absorption or the α-methyl group at C16 influencing the rate of metabolism, or both. Assessment of fibre shedding at day 49 indicated little difference between the three steroid derivatives; however, the dense wool regrowth on betamethasone-treated sheep following fleece plucking was a desirable result as it would provide some protection against effects of climate.

Three of the four sheep treated with triamcinolone acetonide showed a similar response to those treated with betamethasone, suggesting that methyl or acetonide radicals at C16 are both associated with the defleecing activity of a steroid. Florini *et al.* (1961) suggested that the metabolism of triamcinolone in the dog by slow 6β-hydroxylation is a result of the inhibition of other pathways of metabolism by the presence of the 9α-fluoro and 16α-hydroxyl groups. Betamethasone is also metabolized in man by a similar transformation (Butler and Gray 1970). The apparently similar defleecing activity of betamethasone and triamcinolone acetonide may reflect similar affinities for peripheral receptors or alternatively suggests that, in addition to decreasing the rate of absorption, the 16,17-acetonide in triamcinolone replaces the influence of a 16α-methyl group in the metabolism of betamethasone.

One sheep treated with triamcinolone acetonide showed no depression in linear wool growth and yet showed brush end formation and fibre shedding. The expression of defleecing activity without depression in linear wool growth is an unusual, but desirable phenomenon and warrants further investigation of this response. However, the hyperactive linear wool growth seen in this sheep and others in these experiments may not indicate increased wool production because Chapman and Bassett (1970) reported increased fibre length growth rate and concomitant reduced clean wool production following cortisol injection.

Numerous breeds of sheep have the capacity to shed their fleece seasonally. This may have been achieved through the influence of a specific catagenic adrenocortical steroid which was responsible for the formation of brush end fibres. Merino sheep have lost the capacity to shed their fleece and this is probably due to selection against this characteristic in the development of the breed. The concept of an adrenal steroid which has a specific role of inducing fibre shedding is further supported by the observation of Chapman (1980) who reported that brush end fibres produced by synthetic corticosteroids are similar to, although smaller than, naturally shed fibres. The similar defleecing response to triamcinolone and betamethasone and the results of Sayers and Travis (1970) showing the comparative anti-inflammatory potency of triamcinolone and betamethasone as 5 and 25, respectively, suggest little relationship between defleecing and anti-inflammatory activities. These comparisons introduce

the possibility of a corticosteroid which has potential to cause fibre shedding without anti-inflammatory activity. Even betamethasone appears to have a relatively low activity for the induction of fibre shedding in sheep when it is considered that dose rates of 36–132 mg/40 kg liveweight are required to induce fibre shedding, while only 8–16 mg are required to induce parturition in ewes after 137 days of pregnancy. The potential of a specific catagenic steroid which produces brush end fibres warrants further investigation of the defleecing activity of synthetic corticosteroids with variations in molecular structure based on the principles established in the present experiments.

The effect of three daily i.m. injections of the microcrystalline suspension of betamethasone alcohol is reproduced by an 8-day infusion of soluble betamethasone phosphate and the response is evidently dose-dependent. Long-acting injections therefore appear to produce similar defleecing effects to those of long-term infusion. The results of experiments 3, 4 and 5 showed the MEDD of betamethasone administered as three equally divided daily injections or as a single i.m. injection to be approximately 2·1 mg/kg. The fibre-shedding and fleece-casting response to this dose rate showed considerable variation as assessment included categories 1, 2 and 3. The wool on the head and neck of those sheep that responded to single i.m. injections of 0·3, 0·9 and 1·5 mg/kg betamethasone could not be plucked by hand and 7 of the 18 sheep treated with these dose rates showed little or no shedding response on the body. Although it has been demonstrated that a single i.m. injection of betamethasone produces fibre shedding, these observations emphasize the need to find a synthetic corticosteroid which has a more specific defleecing activity than that of betamethasone.

The administration of glucocorticoids results in increased blood glucose, a rise in liver glycogen and increased resistance to insulin (Sayers and Travis 1970). The results of experiments 6–9 suggest that the gluconeogenic role of betamethasone has little effect on fibre shedding. The simultaneous administration of betamethasone and glucose appeared to have an additive effect on depression in linear wool growth without influencing shedding. This suggests that increased hyperglycaemia does not enhance the catagenic effect of the steroid; however, it may influence the associated depression in wool growth.

Kalhan and Adams (1975) showed that glucose is an important physiological stimulus for insulin release. However, treatment with prednisone appeared to inhibit the pancreatic insulin response to hyperglycaemia. In the present experiments concurrent treatment with insulin did not appear to alter depression in linear wool growth or fibre shedding caused by 3·3 mg/kg betamethasone. This indicates that insulin insufficiency is unlikely to be a limiting factor in wool growth depression or brush end formation at this dose rate of betamethasone. The results of experiment 8 also illustrate that sheep treated with 2·1 mg/kg betamethasone are not clearly as resistant to insulin as are those treated with 3·3 mg/kg betamethasone. Kahn *et al.* (1978) showed that glucocorticoid excess resulted in insulin resistance and a marked decrease in insulin binding to its receptors. This decrease in binding appeared to be due to a decrease in insulin sensitivity observed in the presence of excess steroid, and was accounted for, at least in part, by alterations in insulin-receptor interaction. The lack of response to insulin treatment in the present work may have resulted from this reduction in receptor affinity due to treatment with 3·3 mg/kg betamethasone.

Sayers and Travis (1970) cite the use of chlorpropamide for insulin replacement therapy in the treatment of patients suffering from maturity onset diabetes who are

readily controlled with less than 20 units of insulin per day. Chlorpropamide acts by stimulating pancreatic secretion of insulin. In the present work chlorpropamide administration appeared to affect the linear wool growth response to betamethasone at 2·1 mg/kg but not at 3·3 mg/kg. This again demonstrates the insulin resistance of sheep treated with 3·3 mg/kg betamethasone and suggests that sheep treated with 2·1 mg/kg betamethasone maintain their insulin sensitivity. The results of experiments 7, 8 and 9 suggest that 3·3 and 2·1 mg/kg betamethasone may suppress endogenous insulin secretion and that the former dose rate elicits a marked reduction in insulin receptor affinity which results in a high resistance to insulin.

Chlorpropamide appeared to prevent the depression of linear wool growth in response to 2·1 mg/kg betamethasone; however, there was little reduction in fibre shedding. This suggests that impaired glucose utilization in steroid-treated sheep may contribute to wool growth depression but is not an essential component of fibre shedding and therefore wool growth depression may not be necessary for brush end formation. These conclusions tend to agree with the results of Panaretto *et al.* (1975) who reported prolonged wool growth depression without fibre shedding and that plasma glucose concentrations following i.v. and i.p. injections of dexamethasone trimethylacetate and i.v. injection of dexamethasone phosphate were not well correlated with responses to wool fibre shedding.

The administration of glucose to sheep did not affect plasma corticosteroid levels; however, insulin caused a fivefold increase in corticosteroid concentration (Bassett and Hinks 1969). Landon *et al.* (1963) reported that an increase in plasma cortisol in response to insulin-induced hypoglycaemia requires an intact hypothalamo-pituitary-adrenocortical (HPA) axis. As the HPA axis in the present experiments would be severely depressed by the high dose of exogenous corticosteroid, elevation of endogenous plasma corticosteroids was unlikely.

Significant depression of endogenous thyroxine during i.v. infusion of dexamethasone phosphate was reported by Panaretto and Wallace (1978*a*). Gordon (1980) administered 27 mg L-thyroxine s.c. per sheep 8 days after i.m. injection of dexamethasone isonicotinate and found no effect on the rate of wool growth, although it was suggested that the lack of response may have been due to poor absorption. In experiment 10 the daily s.c. injection of 300 μg L-thyroxine per sheep administered simultaneously with betamethasone i.m. and for 20 days thereafter had no effect on wool growth. It appears that depression of plasma thyroxine following treatment with these steroids does not contribute as a single entity to wool growth changes.

These experiments indicate that synthetic corticosteroids currently used for therapeutic purposes have limited potential for use as defleecing agents in the wool industry. The results suggest little relationship between therapeutic potency and defleecing activity and emphasize the influence of small changes in molecular structure. These findings provide an opportunity for the development of a synthetic corticosteroid with properties embracing a specific activity for the induction of fibre shedding and an absence of therapeutic effects.

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