Comparisons of the Plasma Steroid Concentration Profiles and Wool Growth Responses after Administration of Two Forms of Dexamethasone to Sheep

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Abstract

Sheep were treated with dexamethasone (1) as the water-soluble 21-phosphate intravenously or (2) subcutaneously, and (3) as the water-insoluble parent compound subcutaneously.

In (1) the infusion was continued for 8 days and plasma concentrations of dexamethasone exceeded 40 ng/ml. The analogue quickly disappeared from the circulation after the infusion was stopped. Compared with pretreatment values wool growth decreased by about 80% 17 days after cessation of the infusion. By 31 days, wool growth was almost back to normal. At this stage most of the fibres that made up the fleece at the time when the sheep were infused had been shed, and it was possible to defleece three of the four treated animals by brushing the wool off manually.

In (2) plasma concentrations of dexamethasone reached very high levels (maximum *c.* 8 μg/ml) but only for a relatively short period (< 48 h). Wool growth was depressed by about 30% 17 days after treatment. No fibre shedding was evident, and the animals could not be defleeced. Recovery in wool growth was almost complete 1 month after treatment.

In (3) plasma concentrations of dexamethasone were in the range of 3–10 ng/ml 15 days after dosage (three out of four animals); dexamethasone could not be detected in the plasma of the fourth animal 12 days after treatment. Wool growth was decreased by about 80% 77 days after steroid administration. Three out of four treated animals could be defleeced manually and in all of them wool growth was depressed 3 months after treatment.

It was concluded from this work that high concentrations of dexamethasone had to be sustained for long periods (8 days) before the majority of sheep could be defleeced manually. A similar result was ultimately achieved following an undesirably prolonged period of wool growth depression, when relatively low concentrations of dexamethasone were sustained for > 15 days. Very high steroid concentration for about 2 days did not have sufficient effect to permit defleecing.

Introduction

Previous papers have described some effects of different forms of dexamethasone (Panaretto *et al.* 1975; Panaretto and Wallace 1978*a*; Leish and Panaretto 1978) and flumethasone (Panaretto and Wallace 1978*b*, 1979) on wool growth in Merino sheep. The results have shown that these steroids induce fibre shedding. However, effects vary between animals and between different body regions in individuals. The inhibition of wool growth after treatment is also variable and occasionally persists for long periods. An earlier study dealt with wool growth and plasma steroid concentrations following the injection of synthetic glucocorticoids by different routes (Panaretto and Wallace 1978*b*). However, no comparisons have been made when animals are treated by the same route with different forms of a particular steroid. The effects of steroids can be significantly modified by the rate at which they are delivered to biological systems. For example, the action of anabolic steroids on the musculature and accessory sex organs of rats is known to vary according to how

readily the drugs enter the circulation after intramuscular or subcutaneous injection (Van der Vies 1965, 1975).

In the present experiment sheep were injected subcutaneously with either water-soluble dexamethasone-21-phosphate or with relatively insoluble dexamethasone. The effects of the two treatments on wool growth were quite dissimilar and the results are interpreted in light of the different patterns of steroid concentrations in the blood. The results of the subcutaneous treatment were compared with the effects of intravenous administration of dexamethasone-21-phosphate.

Materials and Methods

Animals

Sixteen Merino castrate males aged 18–33 months were used. The four animals infused intravenously had fleeces approximately 10 cm in length, while the other 12 had shorter fleeces ranging from 5 to 7 cm. Four of the animals, with a mean body weight ($\pm$ s.e.m.) of $42 \pm 1 \cdot 5$ kg, served as untreated controls. An area of 100 cm² was delineated on the midside of each. All sheep were fed 600 g of a pelleted ration of equal parts of lucerne chaff and oats in one meal daily. The body weights of the animals included the wool; for purposes of calculating the doses of steroids administered to the sheep, body weight raised to the power $0 \cdot 75$ has been used (metabolic body weight, MBW).

Hormone Preparations and Blood Sampling

Dexamethasone (9-fluoro-11β,17-dihydroxy-16α-methyl-pregna-1,4-diene-3,20-dione) as water-soluble 21-disodium phosphate was obtained from Merck, Sharp and Dohme Pty Ltd, Australia, and as dexamethasone from Roussel UCLAF, France. The amounts of dexamethasone administered are given below.

Heparinized blood samples (10 ml) were taken by venipuncture or through venous catheters (see below). Blood samples were always taken on several days before administering dexamethasone. On the day of dosing a sample was taken at 0830 h before the steroid was given and two other samples were taken at 1000 and 1530 h. Blood samples were taken at 0900 and 1530 h daily thereafter. When dexamethasone-21-phosphate was given subcutaneously, blood samples were taken via jugular catheters every half hour and every hour between 0900 and 1600 h on the first and second days respectively. Separated plasma was stored at $-20°C$ until analysed.

Intravenous Infusion

Sheep 1380, 1592, 1567 and 1701, weighing $43 \cdot 1 \pm 1 \cdot 0$ kg ($16 \cdot 8 \pm 0 \cdot 3$ kg MBW), were given $7 \cdot 6$ mg dexamethasone/kg MBW over 8 days as the water-soluble dexamethasone-21-phosphate. One-eighth part of the total dose of dexamethasone-21-phosphate was dissolved in 45 ml saline daily and infused at a constant rate over 24 h using a Harvard infusion pump. Infusion and blood sampling were effected by polyethylene tubing inserted into jugular veins after the method of Panaretto and Wallace (1978a).

Subcutaneous Injections

These were made under the skin over the first three cervical vertebrae. Sheep 2248, 2259, 2264 and 2267, weighing $46 \cdot 6 \pm 1 \cdot 0$ kg ($17 \cdot 8 \pm 0 \cdot 2$ kg MBW), were given $8 \cdot 5$ mg dexamethasone/kg MBW as a single injection of dexamethasone-21-phosphate in 4 ml saline. Sheep 2245, 2252, 2254 and 2256, weighing $45 \cdot 8 \pm 0 \cdot 9$ kg ($17 \cdot 6 \pm 0 \cdot 3$ kg MBW), were given crystalline dexamethasone as a slurry in three injections at the same site, each in 3 ml of saline, at a dose rate of $7 \cdot 7$ mg dexamethasone/kg MBW.

Dexamethasone Assay

Plasma concentrations of dexamethasone were measured by a radioimmunoassay method described by Panaretto and Wallace (1978a) using [³H]dexamethasone (Amersham, England). The antiserum used was prepared at this laboratory and did not cross-react to any significant degree with

the following: cortisol, corticosterone, desoxycorticosterone, progesterone, 17-*OH* progesterone, prednisone, oestradiol-17β, testosterone, and prednisolone. Antiserum was diluted 1/25 000 and standard curves were prepared using progressively doubled concentrations of dexamethasone in pooled sheep plasma ranging from 1·56 to 100 ng/ml; the limit of sensitivity of the assay was 3·1 ng/ml. Standards were assayed in triplicate and unknowns in duplicate; samples with very high concentrations were diluted.

Variations in counts between triplicates, based on the analysis of three standard curves, yielded coefficients of variation (c.v.) in the range 1·2–12·7%, the mean c.v.$\pm$s.e.m. being 4·9$\pm$0·7%. The c.v. for the nine values obtained for counts for any standard concentration, including zero, was 10·7$\pm$2%. The intra-assay c.v. for counts in three assays varied from 3·8 to 5·6% in the concentration range 3·1–>12·5 ng/ml.

Wool Growth

Wool growth was measured by closely clipping the defined areas on the midsides of each sheep every 2 weeks, pretreatment values being obtained during the 3 months before experiment. After treatment, clippings were sometimes made more frequently than every 2 weeks. Wool growth in treated sheep was expressed as a percentage of pretreatment values. Contemporaneous wool growth was measured in four untreated controls. The quantity of clean dry wool was measured in the samples using the method of Hemsley *et al.* (1973).

Wool Shedding

In some experiments a large enough number of wool fibres was shed from follicles to constitute an easily discernible wool 'break' after regrowth was established, but some continuous fibres always remained. Provided they were not too numerous, the continuous fibres could be broken easily by brushing with the hand, and the fleece could thus be harvested. This process is defined as defleecing. In the remaining animals that could not be defleeced a quite marked wool break was sometimes evident.

Results

Plasma Dexamethasone Concentrations

The patterns of plasma steroid concentrations in the three experiments are illustrated in Figs 1*a–c*.

#### (i) *Dexamethasone-21-phosphate, intravenous infusion*

Maximal concentrations, in the range 120–170 ng/ml, were evident during the first 2 days of infusion (Fig. 1*a*). These were followed by concentrations that progressively decreased to 40–60 ng/ml from the 4th to the 6th days of infusion. In three of the sheep this was followed by relatively slight increases in concentration during the final 24–48 h of infusion. Plasma dexamethasone concentrations decreased very quickly once infusion was terminated.

(ii) *Dexamethasone-21-phosphate, subcutaneous implantation*

Dexamethasone entered the circulation very quickly after subcutaneous adminis-tration. Very high concentrations, in the region of 3–8 μg/ml (Fig. 1*b*), were observed within the first 30 min. They then began to decrease and after 24 h the values in three of the four sheep were around 100 ng/ml. By 48–50 h after injection, plasma dexamethasone concentrations in most sheep were below the level of detection.

(iii) *Dexamethasone, subcutaneous implantation*

Plasma dexamethasone concentrations were 25–60 ng/ml during the first 24–48 h after dosing (Fig. 1*c*). Following a decline in concentration, secondary minor peaks
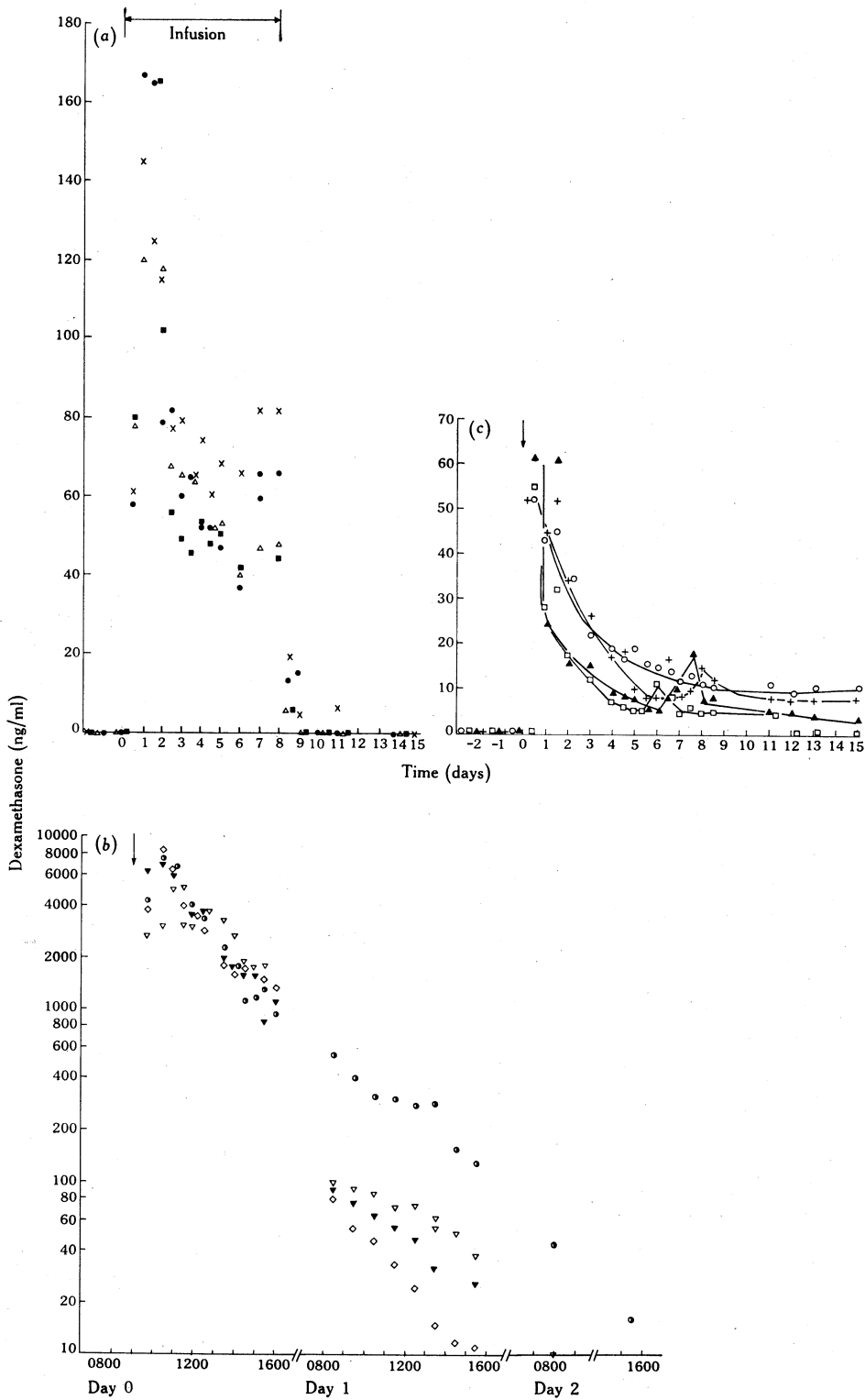
were evident in two or possibly three sheep (sheep 2252, 2254 and 2256; see Fig. 1c) on the 6th to the 8th days after dosage. Plasma concentration decreased to zero in one sheep 12 days after treatment, whereas in the other three animals the concentrations were still 5–10 ng/ml when observations finished 15 days after treatment.

Wool Growth

The soluble form of the steroid when given either intravenously or subcutaneously had a less prolonged inhibitory effect on wool growth compared with the results of subcutaneous treatment with dexamethasone itself.

(i) *Dexamethasone-21-phosphate, intravenous infusion*

Wool growth values after intravenous administration of dexamethasone-21-phosphate are illustrated in Fig. 2a. Seventeen days after treatment the mean ($\pm$ s.e.m.) wool growth decreased to $19 \cdot 6 \pm 11 \cdot 6 \%$ of the mean pretreatment value. The high standard error could be ascribed to the relatively high residual wool growth, 53%, in sheep 1567. The mean of the other three animals was $8 \cdot 3 \pm 4 \cdot 0 \%$ of pretreatment values. By day 45 after treatment the rate of wool growth had almost recovered in all sheep—the mean at this time was $80 \cdot 2 \pm 8 \cdot 9 \%$ of pretreatment values. At this time wool growth in sheep 1567 and 1592 equalled that in untreated controls.

Three out of four sheep (1380, 1592 and 1701) were defleeced approximately 4 weeks after the end of treatment; greasy wool weight was in the range $5 \cdot 0$–$6 \cdot 1$ kg. All defleeced animals were covered by some regrowth. Sheep 1567 coud not be defleeced and wool was removed by clipping the continuous fibres.

(ii) *Dexamethasone-2l-phosphate, subcutaneous implantation*

Wool growth depression in these sheep is illustrated in Fig. 2b. There was little wool growth depression in two of these animals (2259 and 2267) 17 days after treatment. The mean wool growth for the group 17 days after treatment was $72 \cdot 2 \pm 10 \cdot 9 \%$ of the pretreatment mean, i.e. about 30% reduction. The inhibitory effect of the steroid had decreased by day 31 after treatment when the mean wool growth was found to be $88 \pm 5 \cdot 5 \%$ of pretreatment values (Fig. 2b). No fibre shedding was observed in this group. None of the animals could be defleeced.

(iii) *Dexamethasone, subcutaneous implantation*

Wool growth depression was prolonged in this experiment (Fig. 1c), wool growth being almost totally depressed in sheep 2245 and 2252 from day 39 to day 77 after treatment. During the same period wool growth in sheep 2254 and 2256 was depressed to values between 50–60 and 20–30% of pretreatment values respectively. Recovery in wool growth was observed at day 91 after treatment for sheep 2245 and 2252. Wool growth was still depressed in sheep 2254 119 days after treatment, and slowly increased in sheep 2256 from about day 50.

Fig. 1. Plasma dexamethasone concentrations after various treatments. (a) Intravenous infusion of 7·6 mg dexamethasone/kg MBW as dexamethasone-21-phosphate in sheep 1380 (●), 1567 (△), 1592 (■) and 1701 (×). (b) Subcutaneous injection (↓) of 8·5 mg dexamethasone/kg MBW as dexamethasone-21-phosphate in sheep 2264 (▼), 2267 (◑), 2248 (◊) and 2259 (▽). The time of day (h) for the first 2 days after treatment is indicated on the abscissa. (c) Subcutaneous injection (↓) of 7·7 mg dexamethasone/kg MBW as a single subcutaneous depot of dexamethasone in sheep 2245 (○), 2252 (▲), 2254 (□) and 2256 (+).

Sheep 2245, 2252 and 2256 were defleeced 116 days after treatment, and the yield of greasy wool was 3·8–4·8 kg. A small amount of wool (184 g, 4·8% of the total greasy wool removed) on the brisket region of sheep 2256 that could not be removed at defleecing was subsequently clipped off. Because wool regrowth had not started in sheep 2245, some areas of bare skin were left when the fibres were removed from the skin.
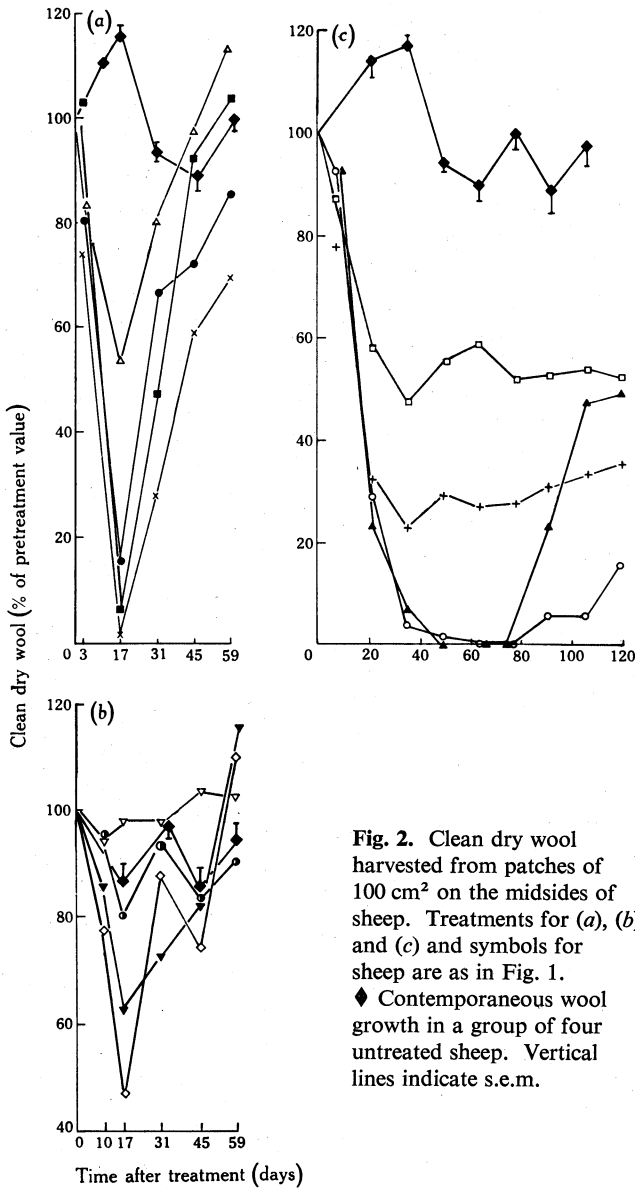

Fig. 2. Clean dry wool harvested from patches of 100 cm² on the midsides of sheep. Treatments for (a), (b) and (c) and symbols for sheep are as in Fig. 1.
♦ Contemporaneous wool growth in a group of four untreated sheep. Vertical lines indicate s.e.m.

Feed Residues

Feed refusals were recorded for sheep 1380, 1567 and 1701 when they were infused with dexamethasone-21-phosphate. Sheep 1380 left four successive residues

in the range 33–65% of its ration during the last 4 days of infusion. Sheep 1567 left five significant residues, in the range 25–68% of its ration, during infusion and for the two following days, while sheep 1701, which had a tendency not to eat all its feed before infusion started, left 80–100% of its ration during the 8 days of infusion and for 5 days after infusion. This animal gradually returned to eating all its food by 10 days after treatment.

Feed residues for animals implanted subcutaneously with crystalline dexamethasone are illustrated in Fig. 3. In three of the animals bimodal patterns of residues were observed, the first peak occurring during the first 2 days after treatment, and the second on days 6–8 after treatment.

There was no refusal of feed by any of the sheep implanted subcutaneously with dexamethasone-21-phosphate.
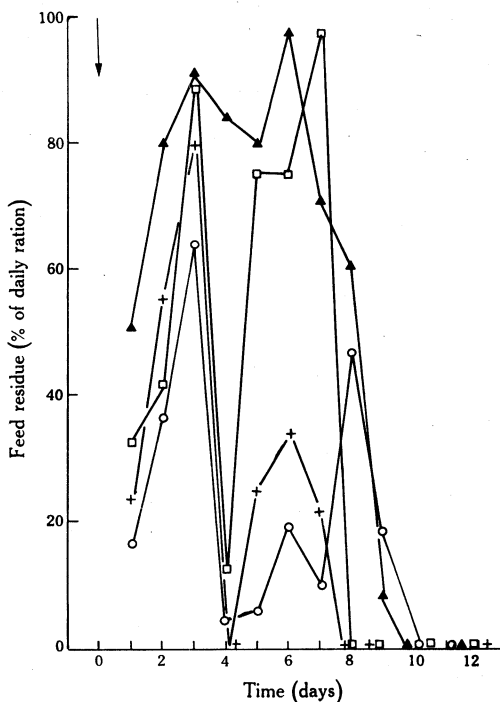

Fig. 3. Feed residues after subcutaneous administration (↓) of 7·7 mg crystalline dexamethasone/kg MBW in sheep 2245 (○), 2252 (▲), 2254 (□) and 2256 (+).

Discussion

In order to defleece the majority of sheep treated with corticoid analogues, it is apparent that concentrations of the steroid in the blood must be maintained at elevated levels for a period of several days. The strategy of reducing the period to 2 days and at the same time increasing plasma steroid concentrations to extremely high values clearly does not affect wool follicles sufficiently to allow the animal to be defleeced. The experiments with subcutaneous treatments showed that the form of the steroid was decisive in determining defleecing activity of the substance. Soluble dexamethasone-21-phosphate was rapidly absorbed and metabolized; it did not remain in the circulation long enough to cause an appreciable weakening of the wool fibres. In contrast, insoluble crystalline dexamethasone was released

relatively slowly from subcutaneous depots and its prolonged presence in the circulation affected fibre growth to an extent that allowed the animals to be defleeced. This same result was achieved by intravenous administration of soluble dexamethasone-21-phosphate for 8 days.

The present work confirms earlier findings (Panaretto and Wallace 1978b, 1979) that depression of wool growth to about 20% of normal values is usually sufficient to enable sheep to be defleeced manually. Insoluble dexamethasone given sub-cutaneously and soluble dexamethasone given intravenously for several days have the common end result of a significant shedding of wool fibres. However, the overall effects of the two treatments on wool growth subsequently were quite different. Subcutaneous administration established plasma steroid concentrations of about 10 ng/ml for long periods (>15 days), and consequently there was a prolonged suppression of wool growth (>90 days). With the intravenous administration plasma concentrations were maintained in the region 40–50 ng/ml for 8 days. Dexamethasone quickly disappeared from the circulation on cessation of the infusion (Leish and Panaretto 1978) and by 45 days after treatment wool growth was almost restored to normal.

The finding that the form of the glucocorticoid influenced its biological activity after subcutaneous administration was in conformity with results reported for other types of steroids. Experiments with anabolic steroids in rats showed that very different biological results were obtained when different forms of the same steroid were injected intramuscularly (Van der Vies 1965). The last author (1975) also demonstrated in rats and sheep that the way in which a steroid is delivered to the body significantly influences its biological effect. Panaretto and Wallace (1978a) have shown that dexamethasone-21-phosphate given intravenously was quickly hydrolysed to free dexamethasone. The present results suggest that the insoluble form of dexamethasone was slowly absorbed from its subcutaneous site whereas the soluble compound was rapidly absorbed as such, hydrolysed to dexamethasone, and quickly metabolized.

While the present experiments provided some clues as to possible causes of prolonged wool growth depression observed after some steroid treatment, they provided no suggestions as to other sources of variability observed (Panaretto et al. 1975). While it might be concluded that failure to defleece sheep 2254 after the sub-cutaneous implantation of crystalline dexamethasone might be ascribed to its lower plasma dexamethasone concentrations, compared with those in the three other sheep, the same could not be said for sheep 1567 following intraveneous infusion. Wool growth on the tattooed patches during the control period in the four sheep that were intravenously infused was in the range 1·8–2·2 g clean dry wool per 14 days, and plasma concentrations of dexamethasone were similar for all four animals, yet wool growth was only slightly inhibited in sheep 1567. These results suggested that the follicles in this animal were not exposed to steroid for a sufficiently long period and/or that the target organs were in some way refractory to the treatment. Whether this resistance to the treatment could be attributed to a defect in the intracellular transfer of dexamethasone to the nuclei in appropriate targets, as was suggested recently in some mouse myeloid leukemic cells (Honma et al. 1977), remains to be seen.

Feed residues in steroid-treated sheep have been reported and some suggestions as to their causes discussed (Panaretto and Wallace 1978b, 1978c). In the present

experiments it was unlikely that the failure to consume all its food influenced the result in any individual with respect to effect on wool growth and subsequent defleecing. For example, when sheep 2254 was implanted with crystalline dexamethasone it left substantial feed residues (Fig. 3) but could not be defleeced. Indeed, this animal showed the least wool growth depression of the four sheep in that experiment (Fig. 1c).

An interesting feature of the pattern of feed residues, illustrated in Fig. 3, was their bimodal distribution. An examination of plasma dexamethasone concentrations (Fig. 1c) in these animals revealed that secondary small increases in concentration of dexamethasone may have occurred on days 6–8 after treatment, so coinciding with the second wave of feed refusals. The causes of feed refusals in steroid-treated sheep have not been investigated here.

Acknowledgments

References

Hemsley, J. A., Reis, P. J., and Downes, A. M. (1973). Influence of various formaldehyde treatments on the nutritional value of casein for wool growth. *Aust. J. Biol. Sci.* **26**, 961–72.

Honma, Y., Kasukabe, T., Okabe, J., and Hozumi, M. (1977). Glucocorticoid binding and mechanism of resistance in some clones of mouse myeloid leukemic cells resistant to induction of differentiation by dexamethasone. *J. Cell. Physiol.* **93**, 227–36.

Leish, Z., and Panaretto, B. A. (1978). Studies on intravenously infused dexamethasone in sheep. *Aust. J. Biol. Sci.* **31**, 373–83.

Panaretto, B. A., Chapman, R. E., Downes, A. M., Reis, P. J., and Wallace, A. L. C. (1975). Some effects of three glucocorticoid analogues on wool growth and their efficacy as defleecing agents in sheep. *Aust. J. Exp. Agric. Anim. Husb.* **15**, 193–202.

Panaretto, B. A., and Wallace, A. L. C. (1978a). Dexamethasone in ovine plasma during its intravenous infusion, its relation to the production of some endogenous hormones and some effects on wool growth. *Aust. J. Biol. Sci.* **31**, 247–55.

Panaretto, B. A., and Wallace, A. L. C. (1978b). The administration of flumethasone by three different routes, its measurement in the plasma and some effects on wool growth in Merino wethers. *Aust. J. Biol. Sci.* **31**, 601–19.

Panaretto, B. A., and Wallace, A. L. C. (1979). Synergistic effects of thiocyanate with flumethasone in inhibiting wool growth in Merino wethers. *Aust. J. Biol. Sci.* **32**, 77–89.

Van der Vies, J. (1965). On the mechanism of action of nandrolone phenylpropionate and nandrolone decanoate in rats. *Acta Endocrinol. (Copenhagen)* **49**, 271–82.

Van der Vies, J. (1975). Drug delivery systems and the biological activity of steroids. *J. Steroid Biochem.* **6**, 215–20.