Some Effects of Dexamethasone on Nucleic Acid Metabolism in Skin of Merino Sheep

B. A. Panaretto,[A] *Z. Leish*[A] *and J. B. Donnelly*[B]

[A] Division of Animal Production, CSIRO,
P.O. Box 239, Blacktown, N.S.W. 2148.
[B] Division of Mathematics and Statistics, CSIRO,
P.O. Box 239, Blacktown, N.S.W. 2148.

Abstract

The effects of 8-day intravenous infusions of dexamethasone on deoxyribonucleic and ribonucleic acid metabolism have been examined in the skin of the Merino sheep.

In two separate experiments, depilatory doses of dexamethasone ($8\cdot4$ mg kg$^{-0\cdot75}$ body weight) were shown to inhibit the [^{3}H]thymidine incorporation into DNA in the skin by about 80%. In the presence of excess thymidine the amount of radioactivity in DNA at the end of infusion decreased to about 50% of the pretreatment values. The incorporation of [^{3}H]uridine into RNA in the skin was not affected.

Decreases in the wet weight, DNA and RNA contents of skin were observed at the end of the dexamethasone infusion. In two experiments, wool growth was reduced to 36 ± 9% (s.e.m.) and 52 ± 8% of the respective pretreatment values.

The results suggest that the dexamethasone caused a reduction in the wool growth by inhibiting DNA biosynthesis of wool follicle cells.

Introduction

Adrenal glucocorticoid hormones or their analogues are known to stimulate or inhibit anagen wool follicles (Chapman and Bassett 1970; Panaretto 1979). In order to induce shedding of the majority of wool fibres, Panaretto and Wallace (1978*a*) and Panaretto (1979) found that it was necessary to maintain elevated glucocorticoid concentrations in the plasma of Merino sheep for 8 days. Although the molecular mechanism of action of this class of chemicals on wool growth remains unknown, several studies with various cells in tissue cultures suggested that the inhibitory effects of glucocorticoids were associated with reductions of the rate of synthesis of deoxyribonucleic acid (Fodge and Rubin 1975; Armelin and Armelin 1977). This may have been due to the arrest of the glucocorticoid-treated cells in the G_1 phase of the cell replication cycle (Harmon *et al.* 1979; Young and Dean 1980).

One method of examining possible modes of action of wool growth inhibitors on nucleic acid and protein metabolism involves incubation of skin slices *in vitro* (Ward and Harris 1976). However, the glucocorticoids, including dexamethasone, were inactive in this system (K. A. Ward, personal communication).

In the present study the rates of incorporation of precursors into nucleic acids in skin from wool-bearing regions in Merino sheep have been measured before, during and after 8-day treatments with depilatory doses of dexamethasone. The prolonged exposure of animals *in vivo* to dexamethasone allows the incorporation of precursors into DNA and RNA to be subsequently studied *in vitro* in skin samples.

Ward and Harris (1976) established that almost 90% of the incorporation of [^{3}H]thymidine into crossbred sheep skin takes place in the rapidly proliferating wool follicle bulb cells. Hence reductions in the [^{3}H]thymidine incorporation into skin DNA during steroid treatment reflect changes in wool follicles. Possible fluctuations in the size of the intracellular thymidine pool which might alter [^{3}H]-thymidine incorporation into DNA were overcome by addition of excess thymidine to the incubation media.

Wet skin weight, DNA and RNA content of skin were also measured throughout the experiments. Relationships between the plasma concentration of dexamethasone, inhibition in wool growth and depilation forces required to pluck wool from steroid-treated animals have been examined.

Materials and Methods

Animals

Twelve Merino wethers, weighing 43–51 kg (Table 1) were housed individually in metabolism cages in an air-conditioned room ($23 \pm 1°C$). The animals used in experiments 1 and 2 were about $3\frac{1}{2}$ and 6 years old respectively. Each animal was offered daily 600 g of a pelleted mixture of lucerne chaff and oats (6 : 4) as one meal at 0900 h. Drinking water was provided *ad libitum*.

Materials

Dexamethasone-21-disodium phosphate (9-fluoro-11β,17α-dihydroxy-16α-methyl-pregna-1,4-diene-3,20-dione-21-disodium phosphate), obtained from Merck, Sharp and Dohme, Australia, was checked for purity by thin-layer chromatography in chloroform : formamide (50 : 1 v/v); [methyl-^{3}H]thymidine (40 Ci mmol^{-1}, 1·48 TBq mmol^{-1}) and [5,6-^{3}H]uridine (47 Ci mmol^{-1}, 1·74 TBq mmol^{-1}), both from Radiochemical Centre, Amersham, U.K., were found, by thin-layer chromatography on silica gel G plates in n-butanol saturated with water, to be 94 and 87% radiochemically pure respectively. Calf thymus DNA, Torula yeast RNA, thymidine and uridine were purchased from Sigma, U.S.A. Eagle's Minimum Essential Medium was supplied by Flow Laboratories, U.S.A.

Intravenous Infusions

Dexamethasone-21-phosphate was dissolved in saline and administered by continuous intravenous (i.v.) infusion into the jugular veins of four (expt 1) and three sheep (expt 2) during an 8-day period at the rates of 1·9 ml h^{-1} using a Harvard infusion pump. The control animals (two, expt 1; three, expt 2) were infused with the vehicle (0·9% NaCl, w/v). Sheep body weights and dose rates are given in Table 1. During the experiment heparinized blood samples (5 ml) were obtained by jugular venipuncture and centrifuged. The plasma was then stored at $-20°C$ for analysis of dexamethasone content.

Skin Sampling

Throughout the experiments four skin samples (total area of 3·14 cm^2) were taken from the left mid-lateral region of the trunk of each sheep at any given time using a circular trephine 1 cm in diameter. The mean pretreatment values here were made prior to the infusion on days -6, -2 and 0 and -7, -5, -3 and 0 in experiments 1 and 2 respectively; the day 0 sample was taken immediately before the treatment began.

The skin samples were cleaned of wool and blood and weighed. They were then cut into approximately 1 mm cubes and separated into homogeneous portions of about 100 mg (expt 1) to be used immediately for incubation with the radioactive precursors of nucleic acids. After extraction and separation, DNA and RNA content and radioactivity were determined (Ogur and Rosen 1950). Nucleic acid contents of sheep skin yielded by this method compared favourably with measurements made using other extraction procedures (Schmidt and Thannhauser 1945; Schneider 1957) and colorimetric analyses (Burton 1956; Ashwell 1957).

Thymidine and Uridine Incorporation

In experiment 1, skin cubes were incubated in 5 ml of Eagle's medium, pH 7·4, at 37°C with 10 μCi of [^{3}H]thymidine or [^{3}H]uridine, in a 95% O_2–5% CO_2 atmosphere for 3 h. The skin samples were then removed from the media, rinsed with cold Eagle's medium, and homogenized with the Polytron PT-10 system for 4×10 s in 3 ml of a solution containing 2 mM EDTA and 1·5 mM Tris-HCl buffer (pH 7·5), as reported by Newman and Cutroneo (1978). The homogenates were combined with $2 \times 0·5$ ml washings of the same solution.

Table 1. Mean body weights $\pm$ s.e.m. of sheep and intravenous doses of dexamethasone given in experiments 1 and 2

Sheep No.	Treatment[A]	Body weight		Total dexamethasone dose		
		(kg)	(kg MBW)[B]	(mg)	(mg kg^{-1})	(mg kg^{-1} MBW)[B]
Experiment 1						
1922, 1927	Dexamethasone	47·5	18·1	150·5	3·2	8·4
1935, 1963		±1·2	±0·3	±3·0	±0·1	±0·2
2260	0·9% (w/v) NaCl	46·8	17·9	—	—	—
2268	0·9% (w/v) NaCl	51·0	19·1	—	—	—
Experiment 2						
9372, 9504	Dexamethasone	45·0	17·3	144·5	3·2	8·4
9678		±1·0	±0·3	±2·4	±0·1	±0·2
9443, 9661	0·9% (w/v) NaCl	46·8	17·9	—	—	—
9734		±2·1	±0·6	—	—	—

[A] All dexamethasone doses are expressed as free dexamethasone.
[B] MBW is metabolic body weight (i.e. body weight$^{0·75}$).

DNA and RNA were isolated from the homogenates using the ethanol, ethanol–ether (3 : 1 v/v), perchloric acid extraction procedure of Ogur and Rosen (1950). RNA was separated from DNA by treating the lipid-free residue with perchloric acid at 4°C for 18 h. DNA was later released from residual protein by heating the samples with perchloric acid. DNA and RNA contents were then determined in the perchloric acid extracts (final concn 1 M) by ultraviolet absorption at 268 and 260 nm respectively.

The [^{3}H]thymidine and [^{3}H]uridine incorporations into DNA and RNA respectively, were determined by counting 1-ml aliquots of ^{3}H-labelled nucleic acid samples in a mixture of 30% (v/v) Brydet X-10 (Bryce and Co., Australia), and toluene-based scintillant containing *p*-terphenyl (0·3% w/v) and 1,4-di[(4-methyl-5-phenyloxazol-2-yl)]benzene (0·01% w/v). Labelled n-hexadecane was used as an internal standard to correct all samples for quenching.

In experiments 2a, 2b, and 2c, skin cubes (a, b = 100 mg; c = 70 mg) were incubated in 2 ml of Eagle's medium, pH 7·4 at 37°C with:

    (*a*) 10 μCi of [^{3}H]thymidine,
    (*b*) 50 μCi of [^{3}H]thymidine + 1·25 μmol of thymidine,
    (*c*) 10 μCi of [^{3}H]uridine

in an atmosphere of 95% O_2–5% CO_2 for 3 h. At the end of incubation the skin samples were removed from media, rinsed in warm (37°C) Eagle's medium (5×2 ml) and stored at -20°C until analysed.

The acid-soluble material was extracted with 0·5 M perchloric acid for 30 min at 0°C (Hagenbuchle *et al.* 1976). The acid-precipitable material was sedimented by centrifugation. DNA and RNA contents and the [^{3}H]thymidine and [^{3}H]uridine incorporation into DNA and RNA respectively were determined as in experiment 1.

Apparent differences in incorporation rates of labels into nucleic acids between experiments 1 and 2 could be reduced to 1 : 2–3 when several variables, including the volume of the incubation mixture (5 and 2 ml), the fact that the wool growth rate in sheep in experiment 2 was 1·5 times those in experiment 1 and, in the case of uridine, the quantity of material incubated (70 and 100 mg), were taken into account.

Dexamethasone Assay

Plasma dexamethasone concentrations were determined by radioimmunoassay (Panaretto 1979). The limit of sensitivity of the assay was $3 \cdot 1$ ng ml^{-1}, the mean coefficient of variation (c.v.$\pm$s.e.m.), based on the analysis of three standard curves, was $4 \cdot 9 \pm 0 \cdot 7 \%$. The intra-assay coefficients of variation for counts in three assays varied from $3 \cdot 8$ to $5 \cdot 6 \%$ in the concentration range $3 \cdot 1$ to more than 12 ng ml^{-1}.

Wool Growth and Fibre Shedding

Every second week wool growth was measured by removing the fibres with fine animal clippers from defined areas (100 cm^2) of skin on the right midside of each sheep. The clean dry weights of the wool samples were measured using the method of Hemsley *et al.* (1973).

Measurements of the force required to pluck wool staples from the mid-back region of the body were made during experiment 2. Changes in force have enabled us to assess the development of weakness in the fleece of steroid-treated sheep caused by shedding of some of the fibres. Values (N/ktex) were obtained using a Hunter spring force gauge (AccuForce Digital Gage; Ametek. Hunter Spring Division, Hatfield, Pa. 19440, U.S.A.) and measuring the thickness of each staple with calipers (Gordon and Pallister 1981).

The mean pretreatment values were derived from 45 measurements made on each animal over a 3-day period. Subsequent values represent means of five measurements made each time on each sheep.

Statistical Methods

The effects of infusion of dexamethasone on skin wet weight, DNA and RNA content in the skin and [^{3}H]thymidine incorporation into DNA were examined in preliminary analyses using hierarchical analysis of variance. Treatments (dexamethasone, saline) were considered as main effects with a corresponding residual term obtained from the variation between sheep within treatments, while days of the pre-infusion, infusion and post-infusion periods were nested within treatments.

Final analysis involved testing the difference between the mean response of the treated (dexamethasone) group, corrected for the mean of that group in the pre-infusion period, and the mean response of the control (saline) group, corrected for the mean of that group in the pre-infusion period. This was achieved by deriving the appropriate standard error of the difference (s.e.d.) between corrected means from each of the above analyses. This comparison, hereafter referred to in this paper as 'the difference between the steroid-treated sheep and the control sheep (corrected for the pre-infusion period)' was of major interest towards the end of the infusion period and at the beginning of the post-infusion period.

Results

Plasma Dexamethasone Concentrations

The plasma dexamethasone profiles of the treated sheep in experiments 1 and 2 are shown in Figs 1*a* and 1*b* respectively. After initial peaks (50–100 ng ml^{-1} plasma) dexamethasone levels fell in experiment 1 within the range of 22–44 ng ml^{-1} for the remainder of the infusion (Fig. 1*a*). In experiment 2, initial levels reached between 65 and 82 ng ml^{-1} and between days 2 and 6 fluctuated in the range of 6–33 ng ml^{-1}. During the final 2 days of infusion (expt 2) plasma dexamethasone concentration increased to about 50 ng ml^{-1} in all treated sheep.

Skin Weight

The mean weights $\pm$ s.e.m. of skin samples taken from four sheep in experiment 1 and three sheep in experiment 2 prior to the steroid treatment were $0 \cdot 53 \pm 0 \cdot 02$ g, $n = 12$, and $0 \cdot 41 \pm 0 \cdot 02$ g, $n = 12$, respectively. In the steroid-treated sheep the

skin weight was reduced to $69\pm4\%$ (Fig. 2a) and $77\pm3\%$ (Fig. 2b) of the corresponding average pretreatment values. In experiment 1, the difference in skin weight between the steroid-treated sheep and the control sheep (corrected for the pre-infusion period) was significant at the end of infusion ($P < 0\cdot02$) and at the beginning of the post-infusion period ($P < 0\cdot01$) [s.e.d. = $0\cdot049$]. A similar result occurred in experiment 2 at the end of infusion and the beginning of the post-infusion period ($P < 0\cdot01$) [s.e.d. = $0\cdot031$].

Fig. 1. Plasma dexamethasone concentrations in (a) sheep 1922 ($\circ$), 1927 ($\square$), 1935 ($\bullet$) and 1963 ($\blacksquare$); and (b) in sheep 9372 ($\circlehalfleft$), 9504 ($+$) and 9678 ($\times$) infused for 8 days with $8\cdot4$ mg dexamethasone $kg^{-0.75}$.

On average, the wet skin weight equalled the values for control sheep about 2 weeks after the infusion was completed. However, in experiment 1 (Fig. 2a) wet skin weight had not returned to its pretreatment values even 5 weeks after the end of infusion.

DNA and RNA Content

The mean DNA contents of skin in the steroid-treated sheep during the pre-infusion periods of experiments 1 and 2 were $1\cdot74\pm0\cdot05$ mg/$3\cdot14$ cm^2 ($n = 12$) and $1\cdot5\pm10\cdot08$ mg/$3\cdot14$ cm^2 ($n = 12$), respectively. At the end of infusion DNA

level in the skin fell to about $78\pm3\%$ (Fig. 3*a*) and $80\pm3\%$ of corresponding pretreatment values (Fig. 3*b*). The difference in DNA content in skin between the steroid-treated sheep and the control sheep (corrected for the pre-infusion period) in experiment 1 was significant at the end of infusion ($P < 0\cdot02$) but not significant 3 days after infusion ceased ($P = 0\cdot06$) [s.e.d. = $0\cdot134$]. In experiment 2 this difference was again pronounced at the end of infusion ($P < 0\cdot001$) [s.e.d. = $0\cdot091$].

During the subsequent 2–3 weeks of the post-infusion period DNA content in the skin gradually returned to the pretreatment levels.
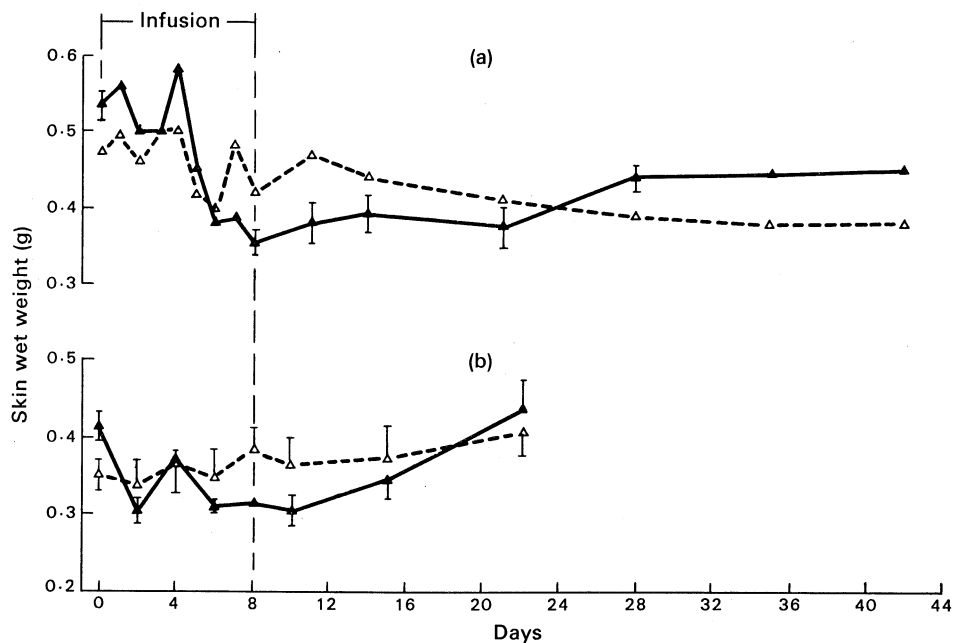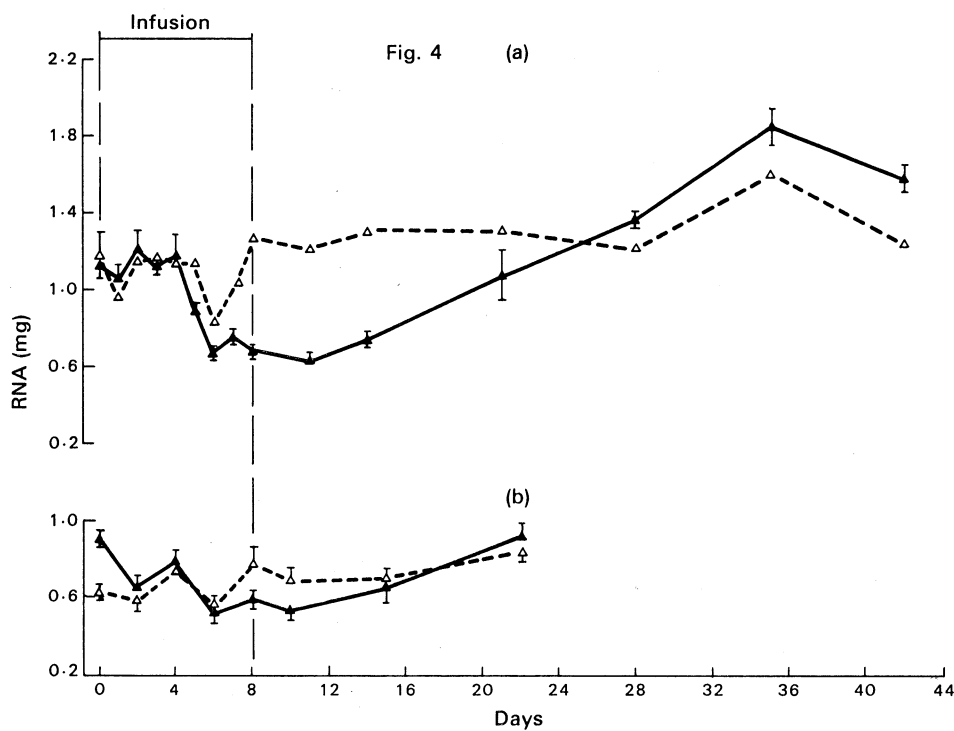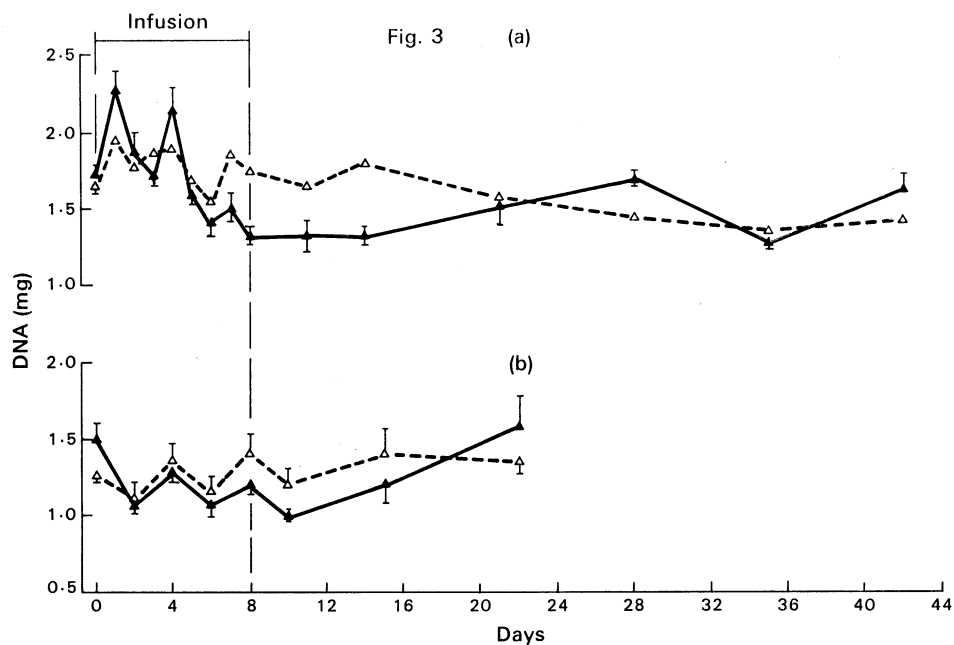

Fig. 2. Effect of the 8-day intravenous infusion of dexamethasone on mean $\pm$ s.e.m. (vertical lines) skin wet weight per $3\cdot14$ cm^2 in (*a*) experiment 1 and (*b*) experiment 2. ▲ Steroid-treated sheep. △ Control. Pretreatment means were established by taking three (expt 1) and four (expt 2) measurements on each animal respectively.

The pretreatment mean skin RNA content for the steroid-treated sheep in experiments 1 and 2 were $1\cdot13\pm0\cdot07$ mg/$3\cdot14$ cm^2 ($n = 12$) and $0\cdot89\pm0\cdot06$ mg/$3\cdot14$ cm^2 ($n = 12$) respectively. During the latter half of the infusion period skin RNA content declined to $57\pm3\%$ (expt 1) and $61\pm2\%$ (expt 2) of the pretreatment values respectively (Figs 4*a* and 4*b*) and reached the pretreatment values after two weeks.

Figs 3 and 4. Effect of the 8-day intravenous infusion of dexamethasone on mean $\pm$ s.e.m. (vertical lines) DNA level (Fig. 3) and RNA level (Fig. 4) per $3\cdot14$ cm^2 of skin in (*a*) experiment 1 and (*b*) experiment 2. ▲ Steroid-treated sheep. △ Control. Pretreatment means were established by taking three (expt 1) and four (expt 2) measurements on each animal respectively.

Fig. 3 (a)

Fig. 3 (b)

Fig. 4 (a)

Fig. 4 (b)

In experiment 1, the difference in RNA content in skin between the steroid-treated sheep and the control sheep (corrected for the pre-infusion period) was not marked until day 8 of infusion ($P < 0\cdot001$) and 3 and 6 days after infusion had ceased ($P < 0\cdot01$) [s.e.d. $= 0\cdot059$]. A similar response occurred in experiment 2 at the end of infusion ($P < 0\cdot001$) and 2 days after infusion stopped ($P < 0\cdot001$) [s.e.d. $= 0\cdot033$].
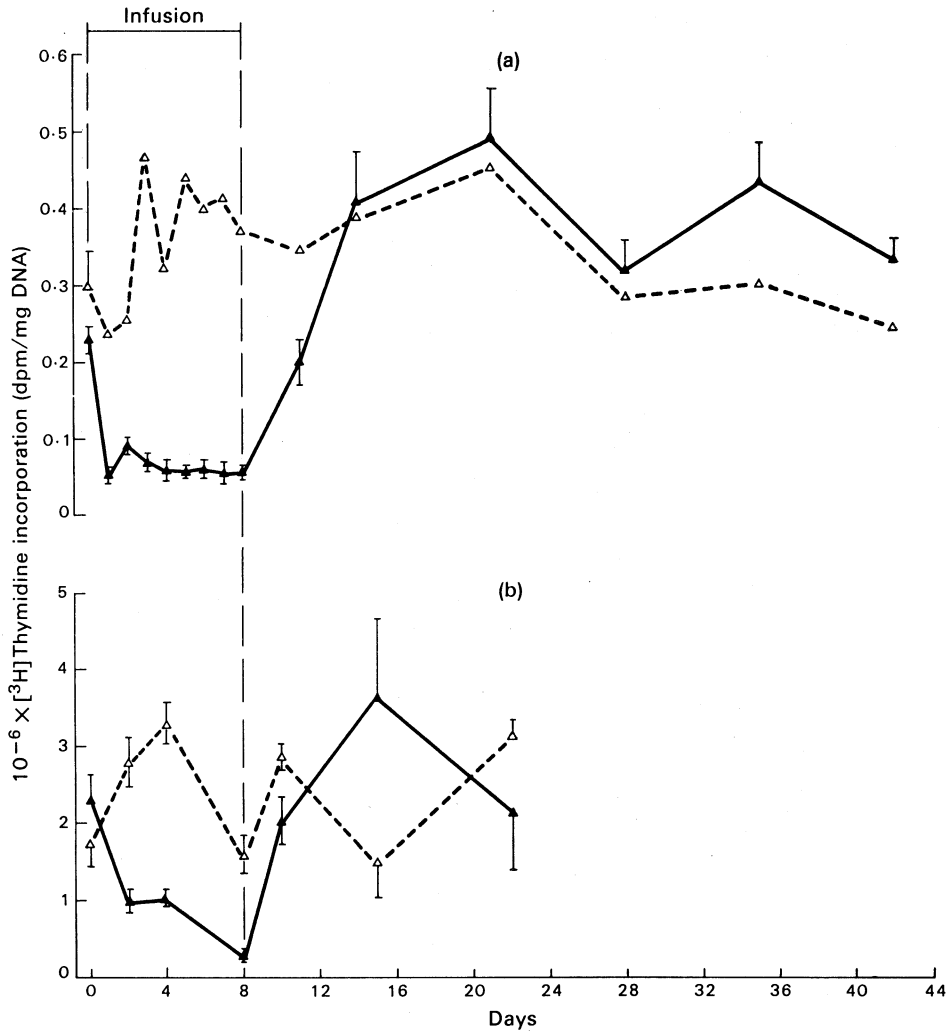

Fig. 5. Effect of the 8-day intravenous infusion of dexamethasone on the mean $\pm$ s.e.m. (vertical lines) [^{3}H]thymidine incorporation into DNA of skin in (*a*) experiment 1 and (*b*) experiment 2. ▲ Steroid-treated sheep. △ Control. Pretreatment means were established by taking three (expt 1) and four (expt 2) measurements on each animal respectively.

The DNA and RNA values expressed on a weight basis remained constant in both experiments, the mean DNA and RNA contents lay in the intervals $3\cdot5$–$3\cdot8$ and $1\cdot8$–$2\cdot5$ mg per gram of skin, respectively, with coefficients of variation of $\leqslant 7\cdot2$ and $\leqslant 17\cdot5\%$.
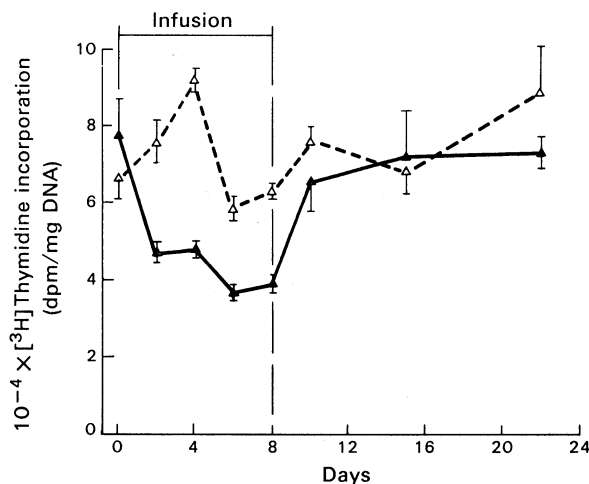
[^{3}H]Thymidine and [^{3}H]Uridine Incorporation into Nucleic Acids

In experiments 1 and 2 the mean pretreatment values of [^{3}H]thymidine incorporation into skin DNA were $2 \cdot 2 \pm 0 \cdot 2 \times 10^5$ and $2 \cdot 3 \pm 0 \cdot 3 \times 10^6$ dpm mg^{-1} DNA respectively. These values were significantly reduced during the infusion period (Figs 5a and 5b) and at the end of treatment reached only between 13 and 25% of pretreatment values. After completion of the infusion, values reached or exceeded the pretreatment level in the next 3 days.

In experiment 1, the difference in [^{3}H]thymidine incorporation in DNA between the steroid-treated sheep and the control sheep (corrected for the pre-infusion period) was highly significant ($P < 0 \cdot 001$) on days 7 and 8 of the infusion period, but this difference was not significant ($P = 0 \cdot 22$) 3 days after infusion had ceased [s.e.d. $= 0 \cdot 573$].

The difference in [^{3}H]thymidine incorporation in DNA between the steroid-treated sheep and the control sheep (corrected for the pre-infusion period) in experiment 2 was significant throughout the infusion period ($P < 0 \cdot 02$) but not 2 days after infusion finished ($P = 0 \cdot 07$) [s.e.d. $= 0 \cdot 736$].

Fig. 6. Effect of the 8-day intravenous infusion of dexamethasone on the mean $\pm$ s.e.m. (vertical lines) [^{3}H]thymidine incorporation into DNA of skin in presence of $1 \cdot 25$ μmol of thymidine in experiment 2. ▲ Steroid-treated sheep. △ Control. Pretreatment means were established by taking four measurements on each animal.

The [^{3}H]thymidine incorporation into DNA of skin in the presence of $1 \cdot 25$ μmol of thymidine in experiment 2 prior to the treatment was $7 \cdot 84 \pm 0 \cdot 9 \times 10^4$ dpm mg^{-1} DNA. In the steroid-treated group the incorporation gradually declined to $48 \pm 4\%$ of the pretreatment level (Fig. 6). Values returned to pretreatment levels in the week following the end of infusion. At the end of the infusion period the difference between the steroid-treated sheep and the control sheep in the amount of [^{3}H]thymidine incorporated into DNA was significant ($P = 0 \cdot 01$), but this difference was not evident 2 days after infusion had ceased ($P = 0 \cdot 13$) [s.e.d. $= 1 \cdot 377$].

The [³H]uridine incorporation into skin RNA in experiments 1 and 2 was $3 \cdot 4 \pm 0 \cdot 4 \times 10^5$ and $6 \cdot 8 \pm 1 \cdot 7 \times 10^6$ dpm mg^{-1} RNA respectively prior to the treatment and was not significantly affected by the treatment.
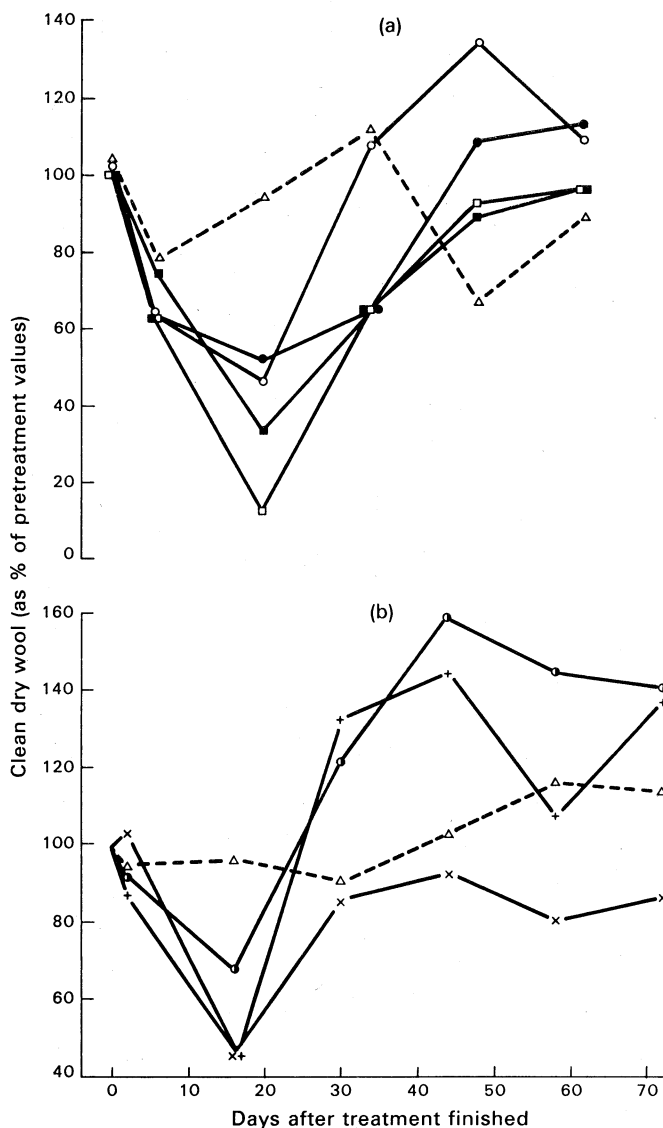

Fig. 7. Clean wool as a percentage of pretreatment values in (a) experiment 1—sheep 1922 (○), 1927 (□), 1935 (●), 1963 (■), and (b) experiment 2—sheep 9372 (◑), 9504 (+) and 9678 (×). △ Mean values for the controls.

Wool Growth

The depression of wool growth following the steroid treatment in both experiments is illustrated in Fig. 7. Values have been expressed as percentages of the means of five (expt 1) and three (expt 2) pretreatment samples taken at fortnightly intervals.

The mean residual wool growth about 20 days after the completion of infusion was 36 ± 9 (expt 1, Fig. 7a) and $52 \pm 8\%$ (expt 2, Fig. 7b) of pretreatment values. Wool growth was restored to its pretreatment levels in 48 days (expt 1) and 25 days (expt 2).
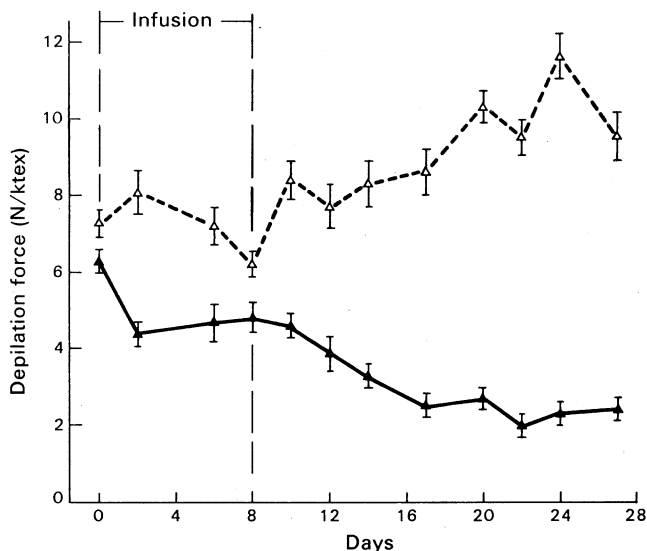

Fig. 8. Mean $\pm$ s.e.m. depilation forces required to pluck wool staples from the midback region of dexamethasone-treated sheep (▲) and controls (△) in experiment 2.
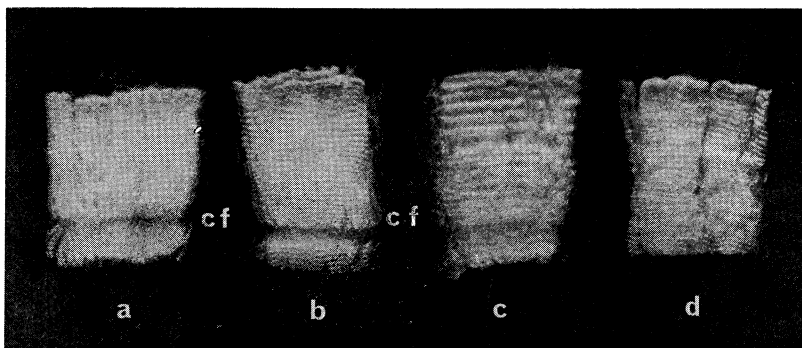

Fig. 9. Wool samples (staple length $7 \cdot 5$ cm) taken from the midback regions of dexamethasone-treated sheep 9372 (a), 9504 (b), 9678 (c) and control sheep 9443 (d) in experiment 2, 2 months after the infusion finished. The less-dense zone in the wool staples a–c were composed of continuous fibres (cf) apparently unaffected by dexamethasone. The wool above this region consisted principally of fibres shed due to dexamethasone treatment together with the continuous fibres. Regrowing fibres mixed with continuous fibres are located below the zone of lower density. No changes were observed in the wool fibres of the control sheep.

The effects of fibre shedding (Fig. 9) in experiment 2 were clearly reflected in the reduction in mean depilation forces required to pluck wool (Fig. 8). Despite the low mean depilation forces $(1 \cdot 9 \pm 0 \cdot 4$ N/ktex) recorded for the steroid-treated sheep 17 days after treatment finished (Fig. 8), the fibres apparently not affected and

therefore not shed were so numerous as to cause retention of the whole fleece. About 1 month after treatment it was still not possible to harvest these fleeces easily by breaking the continuous fibres manually (Fig. 9).

Discussion

Dexamethasone depressed wool growth by 64 and 48% of pretreatment in experiments 1 and 2 respectively. The concentration profiles for dexamethasone here were similar to those of Panaretto and Wallace (1978b). Leish and Panaretto (1978) reported similar concentration patterns using [³H]dexamethasone. The peaks in steroid concentration have not been investigated here. The possibilities that exist to explain the initial peaks have been discussed by Panaretto and Wallace (1978b). The secondary peaks observed during the final 48 h infusion might represent rises in unconjugated steroid particularly in the older sheep used.

The [³H]thymidine incorporation into DNA of skin of Merino sheep was inhibited by at least 50% at the end of the 8-day dexamethasone infusion period, in the presence of excess thymidine (Fig. 6). Exogenous thymidine was added to the medium in experiment 2 to overcome any possible changes in the *de novo* and replication thymidine pools during the steroid treatment. Exogenous thymidine (salvage pool) has been shown to mix freely with the small replication precursor pool. In the presence of exogenous thymidine *de novo* synthesized nucleotides do not enter into this expanded replication pool (Kuebbing and Werner 1975). The greater inhibition of thymidine incorporation in experiment 1 (75–87%) compared with 50% inhibition in experiment 2 may have been caused partly by expansion of the thymidine triphosphate replication pool during the dexamethasone infusion.

Although thymidine can serve as a precursor of DNA, it acts as an inhibitor of DNA synthesis when applied at high concentrations, usually 1–2 mM, to mammalian cells (Bjursell and Reichard 1973). The concentration of thymidine (0·625 mM) used in this study was below this level, but various types of cells differ in their sensitivity to thymidine, thus restricting the intracellular production of deoxycytidine nucleotides (Reynolds *et al.* 1979). However, dexamethasone inhibited precursor incorporation in the skin, even though such a restriction appears to have occurred.

Ward and Harris (1976) showed that about 90% of labelled thymidine incorporation in sheep skin was confined to that portion containing the follicle bulbs. It was highly probable that this was the case in the present experiments and the effects of steroid treatment on precursor incorporation were principally on the replicating cells in the follicle bulbs. The close correspondence in experiment 2 between the depression in mean wool growth (52% of pretreatment value) and mean rate of precursor incorporation into DNA of skin (48% of pretreatment values in the presence of excess thymidine) supported the suggestion that inhibitory glucocorticoid effects on wool follicles are mediated by a reduction in DNA synthetic rate (Leish and Panaretto 1979).

We suggest that the present results with respect to DNA indicated that a block was introduced at some stage of the proliferative cell cycle in the bulbar region of the wool follicle. Our finding that administration of glucocorticoids to sheep resulted in a marked suppression of thymidine incorporation into DNA (Fig. 5) while DNA content of skin of constant area has only decreased by about 20% (Fig. 3) was similar to the observations reported in livers of weanling rats by Henderson *et al.*

(1971). These latter authors showed that administration of cortisone to young rats produced a rapid and striking inhibition of liver DNA synthesis in the absence of an appreciable degradation of DNA and concluded that suppression of net synthesis occurs as a result of suppression of synthesis itself and not of increases in the rate of DNA degradation. Dexamethasone treatment of guinea pig foetuses also significantly depressed the incorporation of thymidine into DNA in various tissues (Sanfaçon et al. 1977).

There were temporal differences in our results between inhibition of label incorporation into DNA (day 2) and reduction in DNA content (days 4–8). Dexamethasone trimethylacetate has been shown to stop mitotic activity in wool follicles during the first 5 days after its injection into Merino sheep (Chapman et al. 1982). The last authors also reported that cells destined to differentiate into the root sheaths and fibre began to withdraw from around the dermal papilla on about day 6. Downes et al. (1966) have shown that [^{3}H]thymidine that was incorporated into cell nuclei during the wool growth in a Merino was lost about 3 days later during keratinization. The reduction of DNA content of skin between days 4 and 8 in skin (Fig. 3) indicated that DNA labelled in wool follicles during dexamethasone treatment here was probably removed as the differentiating inner root sheath and fibre passed up the follicle during the shedding process.

We have shown also a fall by about 40% in RNA content of skin (Fig. 4) during dexamethasone treatment without reduction in uridine incorporation into RNA. This requires further research since, unlike thymidine, uridine incorporation does not occur principally in the dermis (Panaretto, unpublished data). However, liver ribosomal RNA was reduced 33% following prolonged treatment of rats with cortisone with only slight changes in synthetic and degradation rates (Loeb and Tolentino 1970). Macapinlac et al. (1968) showed reductions of RNA in testicular tissues without altered synthesis in the testicular tissues of zinc-deficient rats. The suggestion of these last authors that RNA was being catabolized at an increased rate in such animals was supported by Somers and Underwood (1969). The continuation of RNA synthesis may reflect the differentiation processes in inner root sheath and fibre cells as they withdraw from around the dermal papilla prior to the shedding of fibres (Chapman et al. 1982). Evidence to support this suggestion is also provided by Rovera et al. (1980) who showed cellular differentiation following G_1 blockade of the cell cycle by phorbol diesters, in the absence of DNA synthesis, in cultured human promyelocytic leukaemic cells.

The putative block discussed above may be in the G_1 phase of the cell cycle since a block in this phase has been produced by 60 pg, i.e. $\simeq 200$ ng dexamethasone/ml in several tissue culture studies (Fodge and Rubin 1975; Armelin and Armelin 1977; Harmon et al. 1979; Young and Dean 1980). Kauffman (1977) has reported glucocorticoid-induced withdrawal from the cell cycle of pulmonary epithelial cells in foetal mice.

The mode of action by which glucocorticoids affect DNA biosynthesis and cell division remains unknown. The studies of cultured mouse fibroblasts exposed to steroid for 12 h demonstrated that the treatment inhibited components of the nucleic-acid synthesizing system and not the precursor uptake or conversion to triphosphates (Kemper et al. 1969). On the other hand, Henderson and Loeb (1974) disputed the apparent correspondence between changes in DNA synthetic rate and associated changes in in vitro DNA polymerase activity. They demonstrated that

the effects of glucocorticoids on thymidine incorporation into liver DNA of weanling rats occurred earlier than any detectable effect on polymerase. The fall in thymidine incorporation was also found far greater than the decrease in the enzyme activity.

The role of thymidine kinase, a salvage pathway enzyme for pyrimidine bio-synthesis, as a regulatory function in the control of DNA synthesis during steroid treatment, has not yet been clarified (Sanfaçon *et al.* 1977).

Further studies of DNA polymerase and thymidine kinase activity during the steroid treatment in relation to the wool growth may shed some light on the mechanism of the glucocorticoid mode of action.

Acknowledgments

References

Armelin, M. C. S., and Armelin, H. A. (1977). Serum and hormonal regulation of the 'resting-proliferative' transition in a variant of 3T3 mouse cells. *Nature (London)* **265**, 148–51.

Ashwell, G. (1957). Colorimetric analysis of sugars. In 'Methods in Enzymology'. Vol. III. (Eds S. P. Colowick and N. O. Kaplan.) pp. 73–105. (Academic Press: New York.)

Bjursell, G., and Reichard, P. (1973). Effects of thymidine on deoxyribonucleoside triphosphate pools and deoxyribonucleic acid synthesis in Chinese hamster ovary cells. *J. Biol. Chem.* **248**, 3904–9.

Burton, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**, 315–23.

Chapman, R. E., and Bassett, J. M. (1970). The effects of prolonged administration of cortisol on the skin of sheep on different planes of nutrition. *J. Endocrinol.* **48**, 649–63.

Chapman, R. E., Panaretto, B. A., and Frith, P. A. (1982). Changes in wool follicles of sheep following administration of dexamethasone trimethylacetate. *J. Cell Sci.* **53**, 323–35.

Downes, A. M., Chapman, R. E., Till, A. R., and Wilson, P. A. (1966). Proliferative cycle and fate of cell nuclei in wool follicles. *Nature (London)* **212**, 477–9.

Fodge, D. W., and Rubin, H. (1975). Differential effects of glucocorticoids on DNA synthesis in normal and virus-transformed chick embryo cells. *Nature (London)* **257**, 804–6.

Gordon, A. J., and Pallister, E. (1981). A hand-held device for the rapid measurement of wool staple thickness. *J. Text. Inst.* **72**, 145–6.

Hagenbuchle, O., Schibler, U., and Weber, R. (1976). Incorporation of [5-^{3}H]uridine into ribonucleotide pools and RNA during thyroxine-induced metamorphosis of *Xenopus laevis* tadpoles. *Mol. Cell. Endocrinol.* **4**, 61–77.

Harmon, J. M., Norman, M. R., Fowlkes, B. J., and Thompson, E. B. (1979). Dexamethasone induces irreversible G_1 arrest and death of a human lymphoid cell line. *J. Cell. Physiol.* **98**, 267–78.

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.

Henderson, I. C., Fischel, R. E., and Loeb, J. N. (1971). Suppression of liver DNA synthesis by cortisone. *Endocrinology* **88**, 1471–6.

Henderson, I. C., and Loeb, J. N. (1974). Hormone-induced changes in liver DNA synthesis: effects of glucocorticoids and growth hormone on liver growth and DNA polymerase activity. *Endocrinology* **94**, 1637–43.

Kauffman, S. L. (1977). Proliferation, growth, and differentiation of pulmonary epithelium in fetal mouse lung exposed transplacently to dexamethasone. *Lab. Invest.* **37**, 497–501.

Kemper, B. W., Pratt, W. B., and Aronow, L. (1969). Nucleic acid synthesis in intact nuclei isolated from mouse fibroblasts. Characterization of the system and effects of glucocorticoids. *Mol. Pharmacol.* **5**, 507–31.

Kuebbing, D., and Werner, R. (1975). A model for compartmentation of *de novo* and salvage thymidine nucleotide pools in mammalian cells. *Proc. Nat. Acad. Sci. U.S.A.* **72**, 3333–6.

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

Leish, Z., and Panaretto, B. A. (1979). Effect of intravenously infused dexamethasone on collagen metabolism in skin of Merino sheep. *Aust. J. Biol. Sci.* **32**, 561–74.

Loeb, J. N., and Tolentino, E. M. (1970). Effects of cortisone on ribosomal RNA synthesis in rat liver. *Endocrinology* **86**, 1033–40.

Macapinlac, M. P., Pearson, W. N., Barney, G. H., and Darby, W. J. (1968). Protein and nucleic acid metabolism in the testes of zinc-deficient rats. *J. Nutr.* **95**, 569–77.

Newman, R. A., and Cutroneo, K. R. (1978). Glucocorticoids selectively decrease the synthesis of hydroxylated collagen peptides. *Mol. Pharmacol.* **14**, 185–98.

Ogur, M., and Rosen, G. (1950). The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. *Arch. Biochem. Biophys.* **25**, 262–76.

Panaretto, B. A. (1979). Comparisons of the plasma steroid concentration profiles and wool growth responses after administration of two forms of dexamethasone to sheep. *Aust. J. Biol. Sci.* **32**, 343–51.

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

Reynolds, E. C., Harris, A. W., and Finch, L. R. (1979). Deoxyribonucleoside triphosphate pools and differential thymidine sensitivities of cultured mouse lymphoma and myeloma cells. *Biochim. Biophys. Acta* **561**, 110–23.

Rovera, G., Olashaw, N., and Meo, P. (1980). Terminal differentiation in human promyelocytic leukaemic cells in the absence of DNA synthesis. *Nature (London)* **284**, 69–70.

Sanfaçon, R., Possmayer, F., and Harding, P. G. R. (1977). Dexamethasone treatment of the guinea pig fetus: its effects on the incorporation of ^{3}H-thymidine into deoxyribonucleic acid. *Am. J. Obstet. Gynecol.* **127**, 745–52.

Schmidt, G., and Thannhauser, S. J. (1945). A method for the determination of desoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. *J. Biol. Chem.* **161**, 83–9.

Schneider, W. C. (1957). Determination of nucleic acids in tissues by pentose analysis. In 'Methods of Enzymology'. Vol. III. (Eds S. P. Colowick and N. O. Kaplan.) pp. 680–4. (Academic Press: New York.)

Somers, M., and Underwood, E. J. (1969). Ribonuclease activity and nucleic acid and protein metabolism in the testes of zinc-deficient rats. *Aust. J. Biol. Sci.* **22**, 1277–82.

Ward, K. A., and Harris, R. L. N. (1976). Inhibition of wool follicle DNA synthesis by mimosine and related 4(1H)-pyridones. *Aust. J. Biol. Sci.* **29**, 189–96.

Young, D. V., and Dean, M. C. (1980). The suppression of cellular proliferation in SV40-transformed 3T3 cells by glucocorticoids. *J. Cell. Physiol.* **102**, 223–31.