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Erythemal Response of Biologically Denuded Sheep to Sunlight and the Effects on Skin Structure and Wool Growth

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Abstract

Minimal erythema was produced in the skin of three Merino sheep, which had been denuded with cyclophosphamide, with 1·1 sunburn units of solar erythemal ultraviolet radiation, as measured by a magnesium tungstate detector. The pigmented skin of two Suffolk sheep required 1·7–4·2 sunburn units, depending on the degree of pigmentation. A single dose of 5 sunburn units induced encrustation of the skin 4 days later in both the Merinos and Suffolks.

The anatomical distributions of erythemal ultraviolet over the body of a life-sized model of a sheep were similar at solar altitudes of 69° (February) and 76° (December), and differed significantly from that at 47° (April). The dorsal sites closely approximated horizontal surfaces in the amount of erythemal ultraviolet received. The lateral sites received amounts ranging from <25% of that incident on the dorsal sites at solar altitudes of 69 and 76° up to 50% at 47°.

Consequently, when nine Merino sheep in three groups, which had been denuded with mimosine, *N*-[5-(4-aminophenoxy)pentyl]phthalimide and cyclophosphamide, were exposed continually to sunlight in February at 34°S. latitude, there was a marked dorsoventral gradient in the degree of sunburning. A thick crust formed within the first week on the dorsal and dorsolateral surfaces of the trunk and neck, and a thinner crust on the mid-lateral trunk. Plastic rugs prevented sunburning in other Merino sheep in three groups similarly denuded, while application of 10% (w/v) Friar's balsam in ethanol provided reasonable protection of the exposed areas of the rugged sheep.

The epidermis, upper dermis and the more superficial sebaceous glands of the unprotected sheep became necrotic during the first few days of exposure to sunlight, and were incorporated in the crust which formed on the skin. A new epidermis regenerated beneath this crust and was thicker than normal for 14–20 weeks. Exposure to sunlight also induced increases in overall skin thickness for *c.* 20 weeks and in the sizes of the remaining and regenerating sebaceous glands for *c.* 4 weeks. Sweat glands decreased in size for 3–4 weeks until new ducts regenerated.

Regeneration of the wool follicles in the protected sheep occurred within 4 weeks after dosing with the depilatory compounds, but exposure to sunlight caused a delay of 4–6 weeks in the regeneration of the follicles in the unprotected sheep. Consequently, the latter grew less wool during the first 14 weeks of exposure to sunlight, although this amounted to only 0·2 kg per sheep.

It is estimated that unprotected, denuded sheep near 34°S. latitude would experience substantial sunburn in all months, except May, June and July when exposure on at least two consecutive clear days would be required to produce encrusting sunburn. However, such sheep in the northern wool-growing areas of Australia would sunburn severely throughout the year.

Introduction

When sheep are dosed with a depilatory compound, the effect on the fleece is largely dose-dependent (Gordon and Bennett 1980). There is little loss of tensile strength at low dose rates, development of a weakened zone at intermediate dosages and production of a complete 'break' in the wool at high dose rates. When a break is induced, the fleece is shed leaving the sheep nude. Such animals are very susceptible to cold stress (Gordon

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and Bennett 1980), which can result in death (Roberts and McMahon 1972). Furthermore, in a preliminary study denuded sheep were found to sunburn as readily as untanned Caucasian humans (Gordon and Bennett 1980), and some deaths have been reported at a tropical latitude (Robertson *et al.* 1980). No sunburning was reported by Roberts and McMahon (1972) in a temperate environment. Hence the extent to which sunburn is likely to be a problem in temperate latitudes is unknown. Consequently, further information is presented in this paper on the susceptibility of nude sheep to sunburn, assessed from their erythemal response to solar ultraviolet (u.v.) radiation in a temperate latitude, and the relative quantities of solar u.v. received by various anatomical regions on a sheep are examined. Also presented are details of the pattern of sunburning, the histological changes induced in the skin and the effect on wool growth of denuded sheep exposed to sunlight in midsummer. Using the above information an assessment is made of the likelihood that denuded sheep will sunburn at various times of the year.

Materials and Methods

Erythemal Response of Sheep Skin (Expt 1)

Three medium-wool Merino ewes and two Suffolk ewes were denuded 12–13 days after being dosed in summer with 30 mg cyclophosphamide per kilogram fleece-free liveweight per head, and were either kept indoors or were protected from solar radiation by plastic sheep rugs. On the first cloudless day following defleecing and before regrowth of wool was evident, five areas (80 by 50 mm) of skin on the left dorsal thorax were exposed to mid-day sunlight for periods ranging in 50% increments from 10 to 50 min, and the resulting erythema produced by each exposure was scored independently by two observers after 0, 4 and 24 h. To check more closely on the exposure required to produce minimal erythema, five areas on the right dorsal thorax were exposed on the next cloudless day for periods increasing by 25% throughout that interval in which minimal erythema had previously been produced. The same observers scored the erythemal responses after 0, 4 and 24 h as before. Additional patches were exposed on two sheep for periods up to 150 min to determine the exposure required for crust formation. The intensity of pigmentation and degree of encrustation of the patches were observed for several weeks following each exposure.

Measurements of the erythemally effective solar u.v. radiation were made during the periods of exposure with a magnesium tungstate detector (Robertson 1969) inclined at the same angle to the sun as the test patches of skin. This detector had a relative spectral response to solar u.v. approximating that of human skin and was calibrated in 'sunburn units', one sunburn unit being approximately the exposure required to develop minimal erythema in normal untanned Caucasian humans (Robertson 1972, 1975). From these measurements and the erythemal responses of the exposed patches on the sheep the threshold exposures for minimal erythema and for crust formation were determined.

Anatomical Distribution of Erythemal Ultraviolet Radiation (Expt 2)

The distribution of erythemal u.v. was measured by attaching polysulfone dosimeters (Davis *et al.* 1976) to a life-sized model of a sheep which was cast in polyester resin from a Plaster of Paris moulding of the skinned carcass of a 35 kg Merino ewe. The dosimeters were made of polysulfone film (P1700, Union Carbide Aust. Ltd) of 125 μm thickness cut into 35-mm squares and stapled to 45-mm squares of matt black cardboard. They were attached with contact adhesive to a total of 30 sites on the dorsal, ventral and right side of the model, so that each dosimeter adopted the curvature of the site, with distances between centres of adjacent dosimeters ranging from 45 to 205 mm. The dosimeters on the right side occupied about one-tenth of the surface area of the region. The model was mounted in a standing position on a turntable in a recently grazed paddock. The solar u.v. received by the various body regions was monitored on sunny days with minimal cloud cover for periods of 2 h, symmetrical about solar noon, in February, April and December. The corresponding solar altitudes, calculated from local time by the method of Spencer (1965) were 69, 47 and 76° respectively. During each measurement, the model was rotated 30° every 5 min to simulate various orientations of a sheep to the sun. Similar measurements were made on cloudy days in February and April. Erythemal u.v. was monitored throughout each measurement, as well as at other times (solar altitudes) during the year, in both horizonal and vertical (N., E., S. and W.) planes with the magnesium tungstate detector (Robertson 1969) mounted 0·65 m above the ground surface, equivalent to the height of

the dorsolateral regions of the model. For purposes of calibration, additional dosimeters were exposed on a horizontal surface for periods of 20 min to 2 h. In order to eliminate the effect of variation in erythemal u.v. intensity at the different times of measurement, the dose received by each site on the model was expressed as a percentage of that received by a horizontal surface during the same period of measurement.

The seasonal variation in the daily total erythemal u.v. received by dorsal and mid-lateral regions of unshaded sheep on cloudless days at 34°S. latitude was estimated from the findings of this experiment (No. 2), the daily totals of erythemal u.v., based on calculated hourly solar erythemal radiation at Sydney, N.S.W. (D. F. Robertson, unpublished data), and tables of solar altitude for Sydney (Spencer 1975).

Pattern of Sunburning and the Effects on Skin Structure and Wool Growth (Expt 3)

Three groups each of six medium-wool Merino ewes from the same flock as those in experiment 1 were each dosed orally with depilatory compounds at the following rates per kilogram liveweight:

Group 1	512 mg L-mimosine;
Group 2	338 mg *N*-[5-(4-aminophenoxy)pentyl]phthalimide;
Group 3	28 mg cyclophosphamide.

A complete break was produced in the wool of 17 of the sheep, and when their fleeces were removed manually 13 days after dosing, they were nude, except on the face, ears and lower legs. The wool of the remaining one sheep, which had been dosed with mimosine, was insufficiently weakened to permit complete manual harvesting and the remaining wool was removed by mechanical shearing. Each group of ewes was divided into halves at random. Plastic rugs were put on the sheep in one-half of each group (including the mechanically shorn animal) for a period of 6 weeks, and their uncovered necks, heads and anal regions were painted with 10% (w/v) Friar's balsam in ethanol (benzoin tincture) (G. A. Groves, personal communication). The remaining nude sheep were left unprotected. All the sheep were run together in a paddock without shade at a latitude of 34°S., the exposure to sunlight commencing on 1 February. Because of the very dry seasonal conditions the scant pasture was supplemented with 2–5 bales of lucerne hay per week. The animals were inspected daily for the first 6 weeks and subsequently when weighed (Fig. 4), and were drenched against internal parasites at intervals of 1–2 months.

A skin sample was taken with a 1 cm diameter biopsy punch from the dorsolateral region of each sheep prior to dosing with the depilatory compound. Further skin samples were taken from the same region on each sheep 13 days later when the fleece was removed and exposure to sunlight commenced (i.e. day 0 of exposure), and after 3 and 9 days and 2, 3, 4, 6, 8, 14, 20, 26 and 50 weeks of exposure. The skin samples were fixed in Serra's fluid, dehydrated in increasing concentrations of ethanol and embedded in paraffin. Sections 8 μm thick were cut longitudinal to the follicles and stained with haematoxylin, eosin and picric acid. The sections were examined microscopically to assess the state of the follicles, the thickness of the epidermis as judged by the number of cell layers, the sizes of the sebaceous and sweat glands, the skin thickness, and the extent of cellular infiltration of the dermis and epidermis.

An additional skin sample was taken from the same region of each sheep prior to dosing and at 8, 26 and 50 weeks after the commencement of exposure to sunlight. These samples were fixed in 10% (w/v) buffered formalin and processed into paraffin. Sections 8 μm thick were cut transverse to the follicles at the level of the sebaceous glands and were stained as above. Follicle population density and ratio of secondary to primary follicles (Carter and Clarke 1957) were determined for each sample.

Measurement of Wool Growth (Expt 3)

Dyebands (Chapman and Wheeler 1963) were applied at skin level to the wool on the side and back of each of the 18 sheep at 8, 14, 20 and 26 weeks after commencement of exposure to sunlight. Half of each banded region of wool was removed with fine clippers at 26 weeks to avoid loss of data, due to fading of the distal two bands. The remaining dyebanded wool was removed at 50 weeks. The samples were cut at the base of each band, and the segments of wool were cleaned, conditioned at 20°C and 65% relative humidity and weighed. The proportion of the total wool growth which was grown in each interval was determined after allowance was made for the short pile of wool (1·5 mm) left by the clippers at each clipping.

After 50 weeks of exposure to sunlight, each sheep was given a depilating dose of 25 mg cyclophosphamide per kilogram liveweight. The wool was harvested during the next two weeks and weighed. Samples were taken from the midside region of the fleeces, the percentage clean scoured yields were determined and the clean fleece weights calculated.

The weights of clean wool grown weekly in the periods delineated by the dyebands were calculated for each sheep from its clean fleece weight and the proportionate wool growths averaged for the dyebanded samples from the side and back locations.

Meteorological Records (Expt 3)

Maximum and minimum temperatures in a Stevenson screen and rainfall were recorded daily. A continuous record of total global radiation was obtained from a solarimeter (model CM5, Kipp and Zonen) situated in an adjacent paddock. Measurements of the daily totals of erythemally effective solar u.v. radiation were made with the magnesium tungstate detector (Robertson 1969).

Results

Erythemal Response of Sheep Skin (Expt 1)

Before exposure the skins of the three Merino sheep were pink and visually unpigmented, whereas those of the Suffolk sheep were brown with a variable density of black spots ranging in diameter from 1–3 mm. The minimal erythemal doses of u.v. for the Merinos were 1·0, 1·1 and 1·1 sunburn units and for the Suffolks ranged from 1·7 sunburn units for the least pigmented areas to 4·2 sunburn units on the darker areas; erythema could not be detected visually on the black areas. Pigmentation increased only marginally in the Merino skins and to a dark brown colour in the Suffolks. From the series of prolonged exposures, it was found that single doses of 5 sunburn units led to the formation of crust some 4 days later on the skins of both the Merinos and the Suffolks.

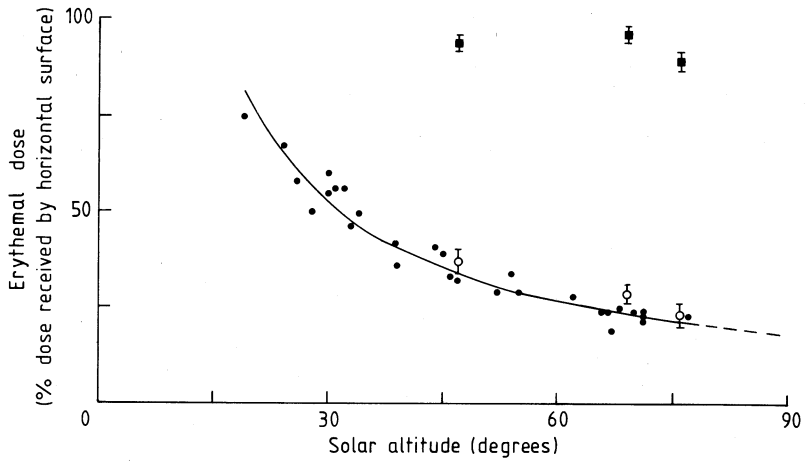


Fig. 1. Relation between erythemal ultraviolet dose, integrated through 360° azimuth, and solar altitude for a vertical plane surface (●) 0·65 m above a grazed pasture throughout the year, and for the means (±s.e.) of four sites on the dorsal trunk (■) and six sites on the mid-lateral trunk (○) of a life-sized model sheep at solar altitudes of 47, 69 and 76°. Cloud cover less than 2 oktas.

Anatomical Distribution of Erythemal Ultraviolet Radiation (Expt 2)

The amounts of erythemal u.v. incident on a horizontal surface on days with minimal cloud cover (0–1 oktas) at solar altitudes of 47° (April), 69° (February) and 76° (December) were 5·0, 9·4 and 9·0 sunburn units in 2 h respectively. On cloudy days (3–6 oktas) at

altitudes of 47° and 69° the incident erythemal u.v. on a horizontal surface amounted to 2·4 and 6·4 sunburn units in 2 h respectively.

The erythemal dose received by a pair of dosimeters of similar orientation and curvature on the dorsal thorax of the model sheep agreed within 8%. There was a consistent ranking of sites in erythemal dose on the sunny and cloudy days at the different solar altitudes, the lowest rank correlation between days being +0·947.

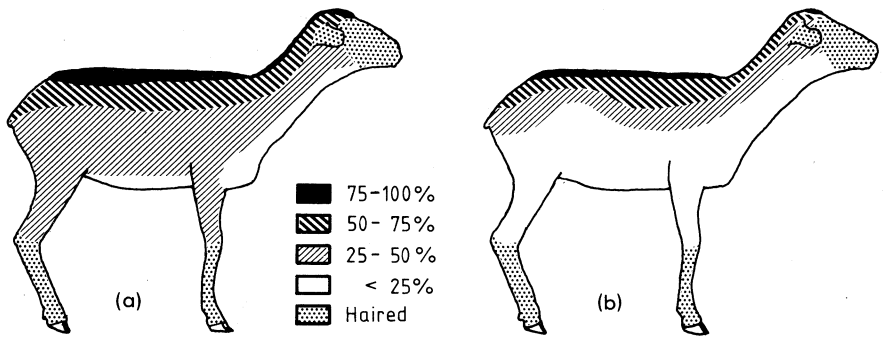


Fig. 2. Distribution of erythemal ultraviolet over the surface of a life-sized model sheep on clear days at solar altitudes of 47° (*a*) and 69–76° (*b*). The ultraviolet intensities are expressed as percentages of the erythemal dose received by a horizontal surface. The effect of solar azimuth was removed by rotating the model 30° every 5 min for 2 h.

The relation between erythemal u.v. dose falling on a vertical plane surface on clear days throughout the year (integrated through 360° azimuth and expressed as a percentage of the dose incident on a horizontal surface) and solar altitude is shown in Fig. 1. Also shown are the relations for the mean values for four sites on the dorsal trunk and six sites on the mid-lateral trunk of the model sheep on clear days at solar altitudes of 47, 69 and 76°. The dorsal sites closely approximated a horizontal surface in the amount of erythemal u.v. received, while the mid-lateral sites were similar to a vertical surface.

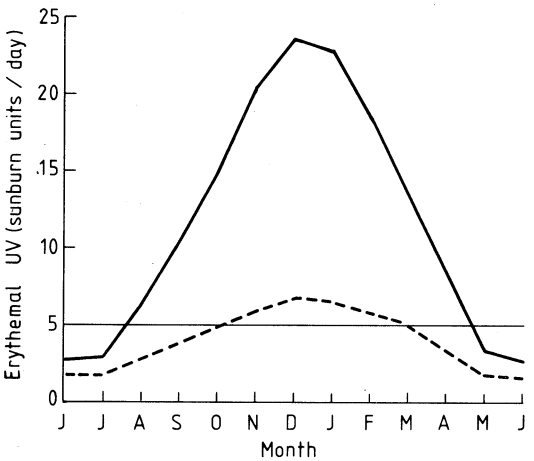


Fig. 3. Seasonal variation in daily total erythemal ultraviolet from clear skies at 34°S. latitude on dorsal (———) and mid-lateral (– – –) surfaces of a sheep. Doses were calculated from the diurnal variation in erythemal ultraviolet for Sydney (D. F. Robertson, unpublished data), tables of solar altitude (Spencer 1975) and Fig. 1. A dose of 5 erythemal units is sufficient to induce encrustation of the skin.

The distributions over the body of the model sheep of erythemal u.v. on clear days at solar altitudes of 47 and 69–76° are shown in Fig. 2. The ventral sites received only 2–6% of the dose incident on a horizontal surface. Considering all sites, the anatomical

distributions at solar altitudes of 69 and 76° were not significantly different, but both differed ($P<0\cdot01$, paired t-tests) from the distribution at 47° altitude (Fig. 2).

Fig. 3 shows the seasonal variation in the daily total erythemal u.v. received by the dorsal and mid-lateral regions of a sheep on clear days at 34°S. latitude. The doses were calculated by assuming horizontal surface values for dorsal sites and vertical surface values for mid-lateral sites (Fig. 1), and by using data on erythemal u.v. radiation (D. F. Robertson, unpublished data) and solar altitude (Spencer 1975) at Sydney.

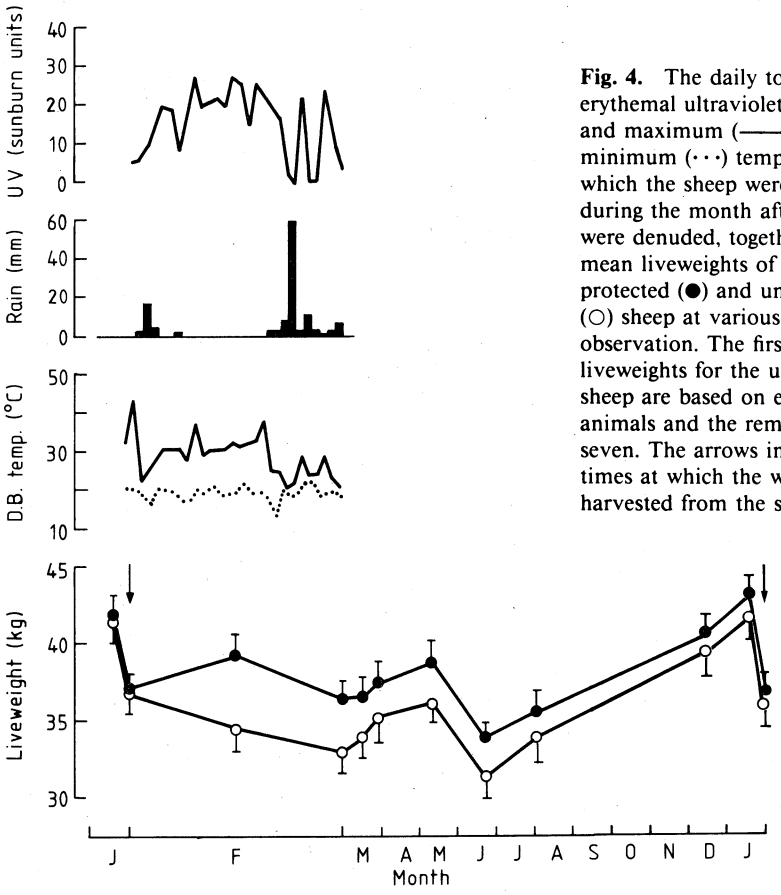


Fig. 4. The daily totals of erythemal ultraviolet, rainfall and maximum (———) and minimum (· · ·) temperatures to which the sheep were exposed during the month after they were denuded, together with mean liveweights of the protected (●) and unprotected (○) sheep at various times of observation. The first five mean liveweights for the unprotected sheep are based on eight animals and the remainder on seven. The arrows indicate the times at which the wool was harvested from the sheep.

Weather Conditions (Expt 3)

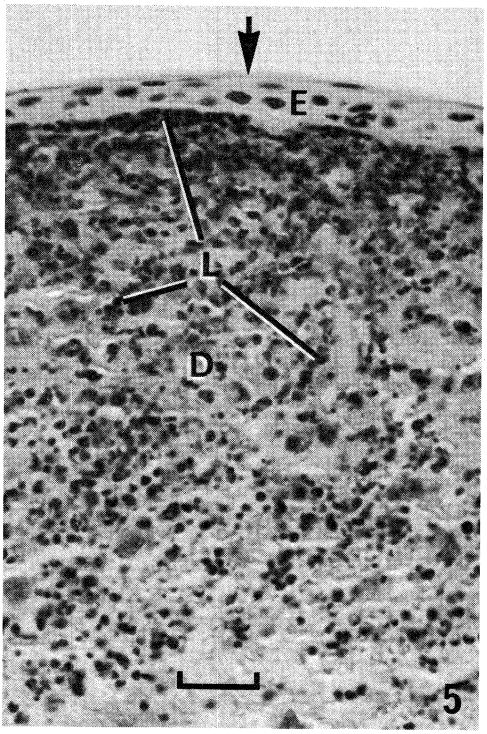
The week prior to the day on which the sheep were defleeced was hot with daily totals of erythemal u.v. ranging from 17 to 28 sunburn units. Similar conditions prevailed while the wool was being harvested from the sheep indoors, but cloud cover increased rapidly from 2 to 5 oktas shortly after the denuded sheep were returned to the paddock. Although dry bulb temperature remained high (up to 43°C), only 5 sunburn units of erythemal u.v. were received by the unprotected sheep during the first day outdoors (Fig. 4). Heavy cloud cover (4–8 oktas) persisted for the next 3 days during which a total of 30 sunburn units of erythemal u.v. and 21·6 mm of rain were received. Only one of the next 14 days was cloudy (8 oktas), and this clear period was followed by 10 days of rain (Fig. 4). Average daily totals of solar radiation and erythemal ultraviolet for the month of February were 18·5 MJ m^{-2} and 16·8 sunburn units respectively.

Table 1. Numbers of cell layers below the stratum corneum in the epidermis of the protected and unprotected sheep prior to and at various times after dosing and denudation with the three compounds

o, Original epidermis; n, new epidermis, but not yet confluent at these times

Treatment of sheep	Time after dosing: 0	13	16	22	4	5	6	8	10	16	22	28	52
	← (days) →				← (weeks) →								
	Time after denudation: 0		3	9	2	3	4	6	8	14	20	26	50
	← (days) →				← (weeks) →								
Sheep dosed with mimosine													
Protected[A]	2–3	2–3	5–7[C]	4–5[C]	3–4[C]	3–6[C]	3–5[C]	3–4	3–4	2–3	2–3	2–3	2–3
Unprotected	2–4	2–5	2–3(o) 3–6(n)[D]	4–10[D]	10–20[E]	10–17[E]	5–17[C]	4–10	4–8	2–4	2–4	2–4	2–4
Sheep dosed with *N*-[5-(4-aminophenoxy)pentyl]phthalimide													
Protected	2–3	4–5	5–7[C]	4–6[C]	4–5[C]	3–4[C]	2–4[C]	2–4	2–4	2–3	2–3	2–3	2–4
Unprotected[B]	2–4	2–4	2–5	3–4(o) 8–15(n)[D]	7–20[E]	6–14[E]	7–14[E]	4–10[C]	3–7	3–5	2–4	2–3	2–4
Sheep dosed with cyclophosphamide													
Protected	3–4	3–4	4–7	3–5	4–6[C]	3–6[C]	3–5[C]	2–4	2–4[C]	2–4[C]	2–4[C]	2–4[C]	3–4
Unprotected[B]	3–4	3–5	3–4(o) 4–5(n)[D]	6–16[D]	12–16[E]	8–15[E]	4–14[E]	4–8[C]	4–6[C]	3–5	2–4	2–4	2–5

[A] Based on the two sheep which were denuded.
[B] Based on the two remaining sheep in the group.
[C] Moderately thick stratum corneum.
[D] No stratum corneum.
[E] Very thick stratum corneum.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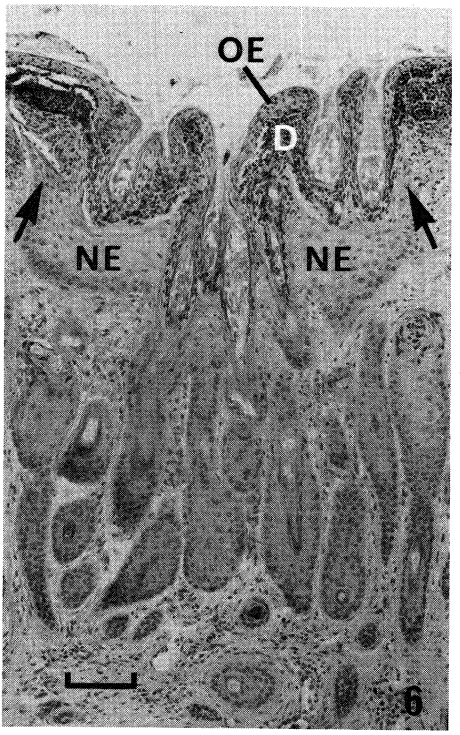
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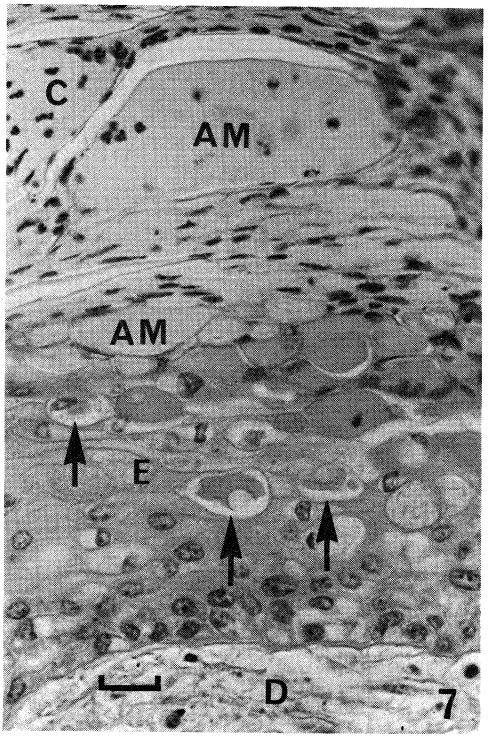
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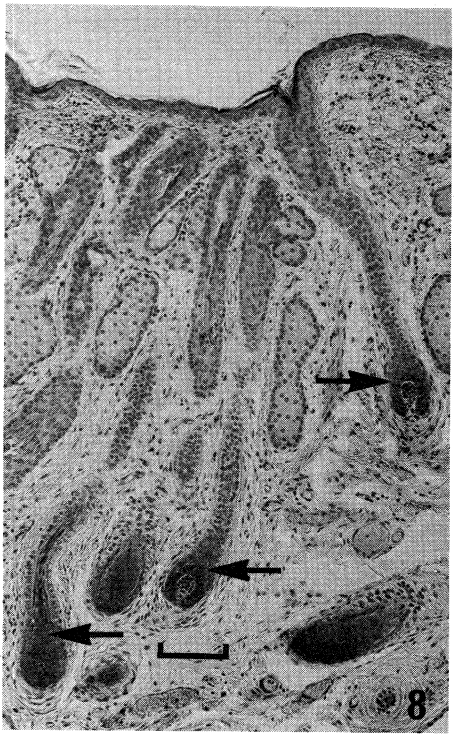
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Pattern of Sunburning (Expt 3)

There was a marked dorsoventral gradient in the intensity of sunburning as indicated by the nature and extent of the crust which formed on the unprotected sheep. A thick crust formed on the dorsal and dorsolateral surfaces of the neck and trunk while thinner crust covered the mid-lateral trunk. Patchy light crust extended on to the ventrolateral trunk and upper legs of six of the nine sheep, and on to the ventral neck of three sheep. The ventral trunk was free from crust. There was no indication of any difference in photosensitivity among the groups denuded by the different compounds. Sunburning did not occur under the rugs on the protected sheep and only a thin crust formed on the dorsal surfaces of the regions painted with Friar's balsam.

Liveweight Changes and Survival (Expt 3)

The vision of three sheep was impaired within 48 h of dosing with *N*-[5-(4-aminophenoxy)pentyl]phthalimide; two were slightly affected for 3 days and the third was more seriously affected for 5 days. The fleece-free liveweight of this third sheep decreased greatly by 5·7 kg between dosing and defleecing and a further 5·9 kg while unprotected in the month following defleecing. She became weaker and progressively hypothermic during the prolonged rainy period, and was killed when transfer to shelter did not relieve the hypothermia and prevent food refusal. Her data were not included in the analyses of liveweight. Another unprotected sheep in the group which had been dosed with cyclophosphamide was removed from the experiment 6 weeks after defleecing when it developed foot abscess. There were no differences in the effects of the three denuding agents on liveweight responses. The mean liveweights of the protected and unprotected sheep are shown in Fig. 4.

The protected sheep gained 2·4±0·5 kg in the first 2 weeks of exposure to sunlight and the unprotected sheep lost 2·4±0·7 kg. Thus the effect of exposure in the first 2 weeks is estimated as a loss of 4·8±0·8 kg. In the following 2 weeks, with rain and low temperatures, the protected sheep lost 2·8±0·3 kg which was greater than the 1·5±0·4 kg lost by the unprotected sheep ($P<0·01$ using Student's *t*-test). It is not possible to separate the effect on liveweight of the setback during this cold period from any continuing effect of exposure to sunlight.

Effects of Exposure to Sunlight on Skin Structure (Expt 3)

Epidermis and dermis

Before the sheep were dosed with the depilatory compounds the epidermis was only 2–4 cell layers thick beneath a very thin stratum corneum, and contained relatively little

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Fig. 5. Vertical section of the upper part of skin of an unprotected, denuded sheep after exposure for 3 days to sunlight. The cells of the epidermis (*E*) have pycnotic nuclei, little stratum corneum is present (→), and leucocytes (*L*) have infiltrated the upper dermis (*D*). Haematoxylin, eosin and picric acid staining. Scale = 20 μm.

Fig. 6. Vertical section of skin from an unprotected, denuded sheep after exposure for 9 days to sunlight. Outgrowths of cells (→) from the distal parts of follicles are forming a new epidermis (*NE*) beneath the necrotic upper dermis (*D*) upon which the old epidermis (*OE*) is still present. Haematoxylin, eosin and picric acid staining. Scale = 100 μm.

Fig. 7. Vertical section of new epidermis (*E*) below portion of the crust (*C*) of necrotic upper dermis of an unprotected, denuded sheep after exposure for 9 days to sunlight. Some of the epidermal cells are vacuolated (→) and no keratohyalin is present. Deposits of amorphous material (*AM*) are located in the lower part of the crust. *D* = dermis. Haematoxylin, eosin and picric acid staining. Scale = 20 μm.

Fig. 8. Vertical section of skin from a sheep at the time the wool was removed 13 days after oral dosing with mimosine, showing follicles in early stages of regeneration (→) but not yet producing keratinized fibres. Haematoxylin, eosin and picric acid staining. Scale = 100 μm.

Table 2. Mean skin thickness (as a percentage of the pretreatment thickness) of the protected and unprotected sheep prior to and at various times after dosing and denudation with the three compounds

Values in parenthesis include the thickness of the crust on the samples

Treatment of sheep	Time after dosing: 0	13	16	22	4	5	6	8	10	16	22	28	52
	← (days) →				← (weeks) →								
	Time after denudation: 0		3	9	2	3	4	6	8	14	20	26	50
	← (days) →				← (weeks) →								
Sheep dosed with mimosine													
Protected[A]	100	67	90	110	110	110	110	110	116	116	110	83	90
Unprotected	100	66	77	73	113	96	100	103	106	100	90	86	90
				(90)	(133)								
Sheep dosed with *N*-[5-(4-aminophenoxy)pentyl]phthalimide													
Protected	100	60	77	100	115	115	115	123	123	115	108	92	108
Unprotected[B]	100	67	85	85	100	104	115	104	104	104	85	88	93
				(96)	(126)	(133)							
Sheep dosed with cyclophosphamide													
Protected	100	73	91	109	118	109	118	118	118	100	100	100	109
Unprotected[B]	100	77	85	88	119	130	104	104	107	107	104	92	104
				(107)	(146)	(157)							

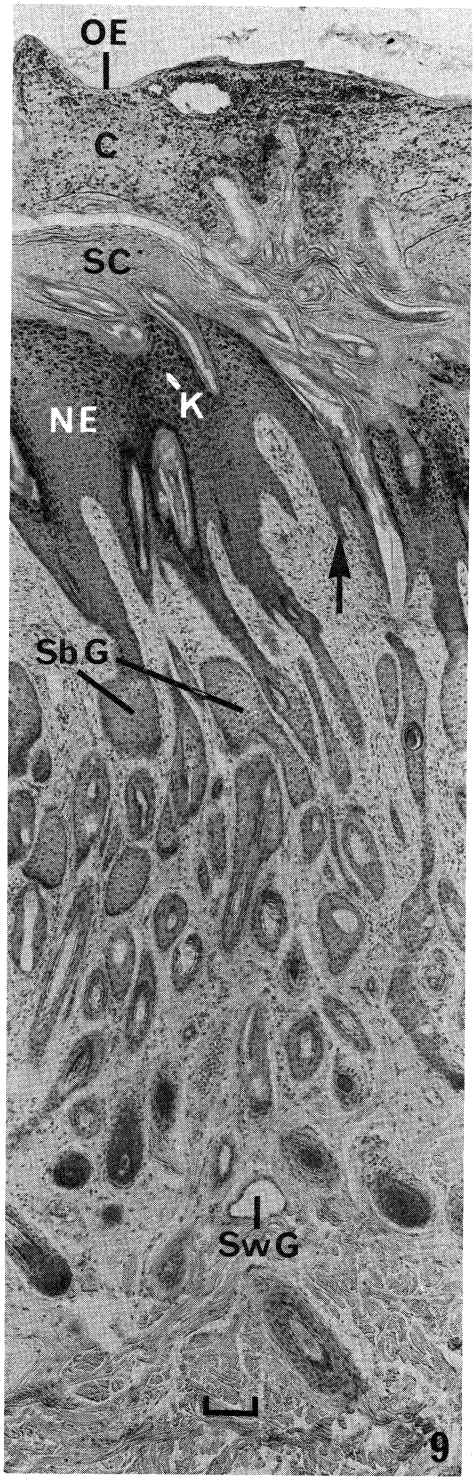
[A] Based on the two sheep which were denuded.

[B] Based on the two remaining sheep in the group.

Table 3. Mean percentages of active follicles and stages of follicle regeneration (after Hardy and Lyne 1956) in the protected and unprotected sheep prior to and at various times after dosing and denudation with the three compounds

Treatment of sheep	Time after dosing: 0	13	16	22	4	5	6	8	10	16	22	28	52
	← (days) →				← (weeks) →								
	Time after denudation: 0		3	9	2	3	4	6	8	14	20	26	50
	← (days) →				← (weeks) →								
Sheep dosed with mimosine													
Protected[A]	100	65	90	100	100	100	100[C]	100[C]	100[C]	100[C]	100	100[C]	100[C]
	F8	F3–F5	F3–F6	F3–F8	F8	F8	F8	F8	F8	F8	F8	F8	F8
Unprotected	100	55	52	55	62	89	100	100	100	100	100	100[C]	100
	F8	F3–F5	F3–F5	F3–F6	F3–F8	F3–F8	F3–F8	F3–F8	F8	F8	F8	F8	F8
Sheep dosed with *N*-[5-(4-aminophenoxy)pentyl]phthalimide													
Protected	100	40	100	100[C]	100	100	100[C]	100	100	100	100	100[C]	100[C]
	F8	F3–F5	F3–F7	F5–F8	F8	F8	F8	F8	F8	F8	F8	F8	F8
Unprotected[B]	100	35	80	70	90	90	97	100	100	100[C]	100	100[C]	100[C]
	F8	F3–F5	F3–F6	F3–F7	F3–F8	F3–F8	F4–F8	F8	F8	F8	F8	F8	F8
Sheep dosed with cyclophosphamide													
Protected	100	60	100	100[C]	100	100	100[C]	100	100	100	100	100[C]	100[C]
	F8	F3–F6	F3–F8	F3–F8	F8	F8	F8	F8	F8	F8	F8	F8	F8
Unprotected[B]	100	50	75	72	75	97	100[C]	100[C]	100[C]	100[C]	100	100	100
	F8	F3–F7	F3–F7	F3–F7	F3–F8	F3–F8	F4–F8	F8	F8	F8	F8	F8	F8

[A] Based on the two sheep which were denuded.
[B] Based on the two remaining sheep in the group.
[C] Remnants of less than 0·5% of atrophied follicles present.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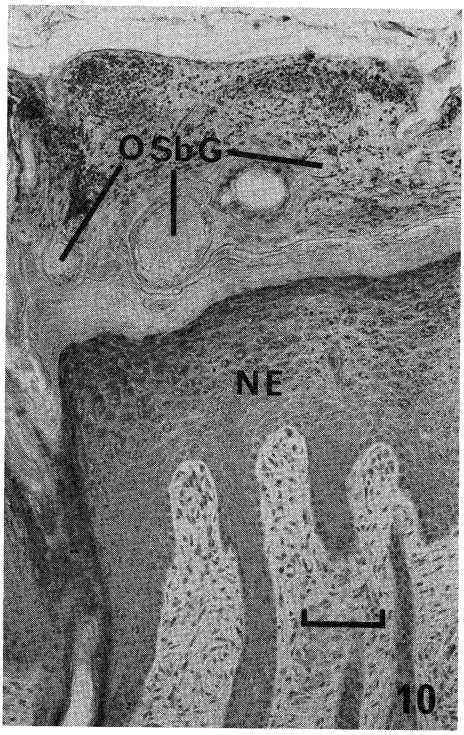
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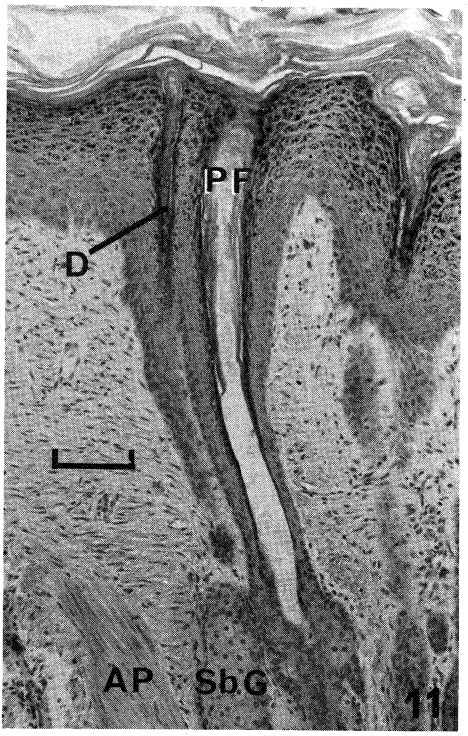
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11

keratohyalin. As indicated in Table 1, the number of cell layers beneath the stratum corneum of the protected sheep approximately doubled after the wool was manually removed at day 13, and subsequently decreased to that prior to dosing during the next 4–6 weeks.

Upon exposure to sunlight the unprotected sheep displayed intense erythema by the end of the first day. At the end of 3 days the epidermal thickness was unchanged, but the nuclei of the epidermal cells were all pycnotic, little stratum corneum was present (Fig. 5) and the skin was becoming hard to the touch. There were some small areas of splitting of the epidermis from the dermis, although severe blistering was not evident. Moderate to intense leucocytic infiltration of the upper dermis was evident after exposure for 3 days to sunlight (Fig. 5), and some leucocytes had invaded the epidermis. Also at this time cellular outgrowths from the outer root sheaths of the distal parts of follicles had started to form a new epidermis beneath that part of the upper dermis which had become necrotic in the unprotected sheep denuded by mimosine and cyclophosphamide (Table 1). Similar outgrowths were present after exposure for 9 days in the unprotected sheep that had been denuded with *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Table 1, Fig. 6). This new epidermis became confluent in 5–6 days, and was grossly thickened, the number of cell layers being *c.* four times greater than before being exposed to sunlight (Table 1). Many of the cells in the newly regenerated epidermis were vacuolated at 9 days after exposure (Fig. 7) and a considerable amount of amorphous material accumulated between the new epidermis and the overlying necrotic dermal tissue. There was little evidence of a granular layer containing keratohyalin in the new epidermis at this time. However, after exposure for 2 weeks there were few vacuolated cells in the new epidermis, which now included both a thick granular layer and a thick stratum corneum (Fig. 9). The new epidermis also had downgrowths into the dermis, some of which appeared to be involved in the regeneration of sweat gland ducts.

The necrotic old epidermis and upper dermal tissue above the new epidermis (Fig. 9) formed a hard crust on the skin surface during the first week of exposure. As the number of emergent wool fibres and the thickness of the stratum corneum increased after 3–4 weeks of exposure, this crust began to lift free of the skin. The new epidermis remained relatively thick during this time, but during the next 10–16 weeks it reverted to the pre-dosing thickness and structure (Table 1).

The overall skin thickness decreased in all sheep after administration of the depilatory compounds, and at the time of manual removal of the wool was about two-thirds to three-quarters the thickness prior to dosing (Table 2). The skin of the protected sheep subsequently became *c.* 10–20% thicker than normal, and remained so until 16–22 weeks after dosing (Table 2). The skin of unprotected sheep also increased in thickness after the wool was removed, but more slowly, and the increase was smaller and less prolonged than in the protected sheep, except in the unprotected cyclophosphamide-treated group (Table 2).

Fig. 9. Vertical section of skin from an unprotected, denuded sheep after exposure for 2 weeks to sunlight. The new epidermis (*NE*) is greatly thickened and has frequent downgrowths (→) into the dermis. Many of the epidermal cells now contain keratohyalin (*K*). A thick stratum corneum (*SC*) has developed and is lifting the crust (*C*) of necrotic upper dermis and old epidermis (*OE*) off the new epidermis. *SbG*, sebaceous glands; *SwG*, sweat gland. Haematoxylin, eosin and picric acid staining. Scale = 100 μm.

Fig. 10. Vertical section of the upper part of skin of an unprotected, denuded sheep after exposure for 2 weeks to sunlight. Some of the original sebaceous glands (*OSbG*) have been damaged by sunlight and incorporated in the crust above the new epidermis (*NE*). Haematoxylin, eosin and picric acid staining. Scale = 100 μm.

Fig. 11. Vertical section of the upper part of skin from an unprotected, denuded sheep after exposure for 4 weeks to sunlight. At this time a regenerated duct (*D*) of a sweat gland emerges directly to the skin surface, and not into the lumen of the primary follicle (*PF*) as normally. *AP*, arrector pili; *SbG*, sebaceous gland. Haematoxylin, eosin and picric acid staining. Scale = 100 μm.

Wool follicles

In 17 of the sheep, 98–100% of follicles were inactivated by the depilatory compounds. When the wool was removed manually 13 days after dosing, there was no sign of any regrowth above the skin surface, although some follicles were in the early stages of regeneration (Fig. 8). In the remaining one sheep only 20% of follicles were inactivated, insufficient to permit manual harvesting of the fleece. The mean percentages of active follicles in the protected and unprotected sheep and the stages of follicle regeneration, based on those described for developing follicles by Hardy and Lyne (1956), at the various times of sampling are listed in Table 3. Virtually all follicles were regenerating in the protected sheep 16 days after dosing with *N*-[5-(4-aminophenoxy)pentyl]phthalimide and cyclophosphamide and 22 days after dosing with mimosine. However, 4 weeks elapsed after dosing before the regrowing fibres emerged from the skin in all follicles of the protected sheep.

Follicle regeneration proceeded more slowly in the unprotected sheep. It was not until 6 weeks after dosing, i.e. 4 weeks after the sheep were exposed to sunlight, that almost all follicles were again active (Table 3). However, 8–10 weeks elapsed after dosing before fibres in all follicles emerged from the skin of the unprotected sheep. A feature of the regenerating follicles in these sheep was a marked thickening of the distal outer root sheaths. This thickening was evident as early as 3 days after exposure and subsided 4–6 weeks later.

Three other follicular changes which occurred with low frequency in the protected as well as the unprotected sheep were distortion of the tips on *c.* 5% of regenerating fibres, atrophy of *c.* 0·5% of follicles and clumping of fibres in the proximal parts of less than 0·5% of regenerated follicles. Distortion of new fibre tips was occurring at the time when the wool was manually harvested and prior to exposure to sunlight. Penetration of follicle walls by some of the distorted tips occurred during the following 4 weeks, after which all fibres emerged normally from the follicles. Because the number of atrophied follicles was so small there was no detectable effect on follicle population density or ratio of secondary to primary follicles. Clumping of fibres was transient and occurred intermittently for 20 weeks after the follicles regenerated.

Sebaceous glands

The sebaceous glands in the protected sheep increased slightly in size after the wool was removed, and remained slightly enlarged for a further 14–20 weeks. In the unprotected sheep some of the more superficial glands decreased in size during the first 3 days of exposure to sunlight, and were subsequently incorporated in the crust above the new epidermis (Fig. 10). From 9 days to 3 weeks after exposure the remaining sebaceous glands in the unprotected sheep were located near the mid-dermal level and were considerably enlarged (Fig. 9). During the next 5 weeks they resumed a level higher in the dermis similar to that prior to dosing, and decreased in size. However, they remained slightly enlarged during the ensuing period of observation, except in the unprotected sheep which had been dosed with mimosine.

Sweat glands

The sweat glands of the protected sheep which had been dosed with mimosine and cyclophosphamide exhibited a transient slight increase in size nine days after these sheep were denuded. No change was detected in the sweat glands of the protected sheep which had been denuded with *N*-[5-(4-aminophenoxy)pentyl]phthalimide. In the unprotected sheep the sweat glands decreased in size and after 9 days of exposure were generally very small (Fig. 9). The distal parts of the sweat gland ducts appeared to be involved in the necrotic changes which also affected the old epidermis and upper part of the dermis. New ducts and orifices could not be identified until after 3–4 weeks of exposure. These new

ducts opened directly to the skin surface through the regenerated epidermis (Fig. 11) and not into the lumen of the primary follicles as usual. With the regeneration of the ducts, the sweat glands increased in size to near that prior to treatment. During the next 5–10 weeks, while the thicknesses of the epidermis and distal walls of the follicles decreased, the orifices of the sweat gland ducts resumed their normal location within the lumen of the primary follicles.

Table 4. Mean annual greasy and clean fleece weights and percentage clean scoured yields of the protected and unprotected sheep

Depilatory compound	Protected sheep			Unprotected sheep		
	Greasy fleece wt (kg)	Clean scoured yield (%)	Clean fleece wt (kg)	Greasy fleece wt (kg)	Clean scoured yield (%)	Clean fleece wt (kg)
Mimosine	3·69[A]	73·7	2·72	3·63	73·7	2·67
N-[5-(4-aminophenoxy) pentyl]phthalimide	3·80	77·0	2·93	4·10[B]	72·1[B]	2·96[B]
Cyclophosphamide	3·75	79·6	2·99	3·92[B]	74·8[B]	2·93[B]

[A] After allowance was made for the amount of wool left on the one animal which was mechanically shorn.
[B] Based on the two remaining sheep in the group.

Effect of Exposure to Sunlight on Wool Growth (Expt 3)

The mean greasy and clean weights and percentage yields of the fleeces grown during the year of observations are listed in Table 4, and the mean weights of clean wool grown

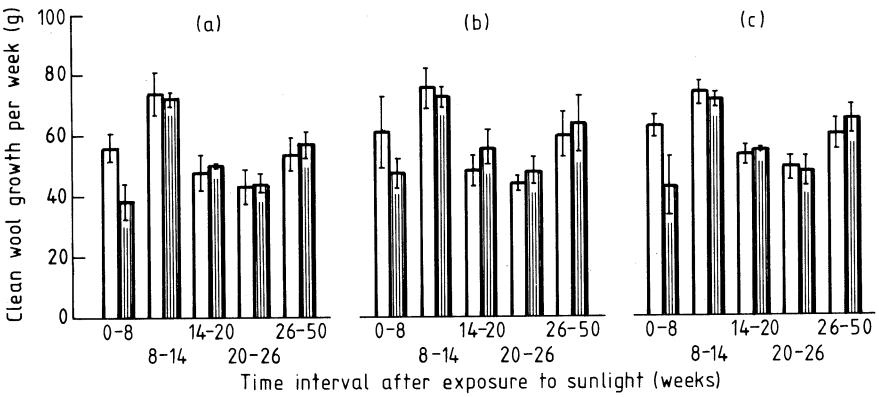

Fig. 12. The mean weights ± s.e. of clean wool grown weekly in the various intervals of time after exposure to sunlight by the protected (open histogram) and unprotected (shaded histogram) sheep, following denudation by mimosine (*a*), *N*-[5-(4-aminophenoxy)pentyl]phthalimide (*b*), and cyclophosphamide (*c*).

per week in the intervals delineated by the dyebands are shown in Fig. 12. In the period from 26–50 weeks after exposure to sunlight there were no differences among the groups denuded by the three compounds. Further, the histological results indicated that the follicles in the unprotected sheep had recovered before 26 weeks. Therefore, using the difference in weekly wool growth during weeks 26–50 from that in each period for each sheep, the effects of exposure to sunlight were tested by comparing the means for the protected and

unprotected groups using one-sided *t*-tests (Table 5). The only significant differences in weekly wool growth between the protected and unprotected sheep were during the first 14 weeks, but the cumulative effect amounted to only 0·2 kg per sheep.

At the end of the period of observation, the only visible difference between the unprotected and protected sheep was the presence on the tips of most of the fleeces of the former of remnants of the crust which had formed during the first week of exposure.

Table 5. Weekly clean wool growth during weeks 26–50 and the differences from the weekly growths in each period for the protected and unprotected sheep

Period (weeks after denudation)	Clean wool weight ± s.e. (g/week) Protected sheep	Unprotected sheep
26–50	57·7 ± 3·2	60·9 ± 3·2
	Differences	
0– 8	2·2 ± 2·3	−18·7 ± 3·5[A]
8–14	16·2 ± 2·1	10·8 ± 3·3[B]
14–20	−8·0 ± 3·0	−7·8 ± 2·3
20–26	−12·5 ± 2·8	−14·5 ± 1·6

[A] Unprotected < protected: $P < 0·001$.
[B] Unprotected < protected: $P = 0·08$.

Discussion

Excessive exposure to sunlight produces many acute and malignant changes in mammalian skin (Blum 1945; Urbach 1969). The high incidence of sunburn and skin cancer induced by the Australian climate and lifestyle (Gordon and Silverstone 1969) has stimulated major programmes to mitigate the undesirable responses of human skin to sunlight (Robertson 1968; Robertson and Groves 1972) and to monitor (Robertson 1968, 1972; Barton and Robertson 1975) or predict (Paltridge and Barton 1978) the distribution of solar erythemal ultraviolet radiation over Australia. One of the intentions of the present experiments was to assess how much of this data could be applied to denuded sheep.

The response of human skin to u.v. depends upon the wavelength of the radiation, the amount of energy received, the degree of pigmentation and the thickness of the epidermis (Blum 1945; Diffey 1982). The responsiveness is maximal at about 295 nm, the shortest wavelength in solar radiation to reach the earth's surface, and falls rapidly to zero at about 320 nm. Multiplication of this spectral responsiveness by the spectral irradiance and integrating the product between 295 and 320 nm provides a measure of the solar erythemal dose. Compounds such as magnesium tungstate (Robertson 1969) and polysulfone (Davis *et al.* 1976) have a spectral response similar to that of human skin and thereby simplify the measurement of erythemal dose. Robertson (1972, 1975) introduced the 'sunburn unit' to calibrate the output of the magnesium tungstate detectors. Minimal erythema in untanned normal Caucasian human skin requires an erythemal dose of approximately 1 sunburn unit. The present study shows that Merino sheep are as susceptible to sunburn as an average untanned Caucasian human, as judged by the 1·0–1·1 sunburn units required to produce a minimal erythema in the three Merino sheep tested. The pigmented, thicker skins of the two Suffolks required 2–4 sunburn units for a minimal erythema. In experiment 1, it was ascertained that a single exposure of 5 sunburn units was sufficient to induce the formation of crust some 4 days later. Severe blistering was not observed in the sheep in any of the experiments, whereas doses of 5–10 sunburn units induce blistering in untanned Caucasian humans (Robertson 1968). So in this regard Merino sheep skin appears to respond differently from human skin.

Once this crust formed, cell migration from the distal outer root sheaths of follicles began to form a new epidermis beneath the crust. This new epidermis initially resembled early fetal sheep epidermis (Lyne and Hollis 1972) in that keratohyalin and stratum corneum were absent. However, the vacuolated cells in the new epidermis were unusual, and the overlying amorphous material may have come from extruded serous fluid. The vacuolated cells were present for only a few days after about 1 week's exposure and were not observed once keratohyalin and stratum corneum were being formed.

The development of the new epidermis evidently took preference over regeneration of the follicles in the unprotected sheep. It was not until near the time when the number of cell layers in the new epidermis started to decrease that virtually all follicles were regenerating. In consequence, it took 4–6 weeks longer for fibres to emerge from all follicles in the unprotected sheep than in those which were protected. Even so, exposure to sunlight did not exacerbate the small amount of follicle atrophy which followed depilation.

Both the sebaceous and sweat glands were affected by ultraviolet radiation, the most superficial sebaceous glands and the distal portion of the sweat gland ducts being destroyed and incorporated in the crust on the skin. But whereas the regenerating sebaceous glands were considerably enlarged, the sweat glands decreased in size until new ducts regenerated. While the epidermis remained grossly hyperplastic, the sebaceous glands were located deeper in the skin than normal and the regenerated sweat gland ducts opened directly to the skin surface through the thick epidermis. Only when the epidermis decreased in thickness did the sebaceous glands and distal sweat gland ducts return to their normal locations.

The present experiments indicate that biologically denuded sheep can survive exposure to sunlight in summer in a temperate latitude (34°S.) without apparent permanent damage to the skin or gross impairment of wool growth. In contrast, deaths (Robertson *et al.* 1980) and scarring (K. W. Entwistle, unpublished observation) have been recorded during exposures to sunlight in a tropical latitude (21°S.). The difference in severity of the two environments appears to be insufficient to account for the disparity in mortality and scarring since the daily total erythemal doses on clear days in summer are 30 and 25 sunburn units for the 21 and 34° latitudes respectively (Paltridge and Barton 1978). Perhaps the less damage in the temperate environment was a consequence of the low erythemal dose of 35 sunburn units received during the cloudy weather of the first 4 days of the present experiment. There is reason to believe that the first 4 days of exposure are the most critical because crust appears on the skin about the fifth day and evidently presents a physical barrier to erythemal radiation, as judged by the proliferation of epidermal cells beneath the crust. Scarring or death might be a consequence of sunburn in a temperate latitude during summer if clear skies were to persist for the first 4–5 days of exposure.

Because of the distribution of erythemal u.v. over the body of the sheep (Fig. 2), it is only the dorsal and lateral regions that require protection against sunburn. Plastic rugs provide adequate protection, but conventional designs do not cover the head, neck and anal region. One application of Friar's balsam (benzoin tincture) provides reasonable protection for up to 9 days, presumably due to the formation of a tenacious film by penetration of the alcoholic solution into the epidermis (Robertson and Groves 1972). Polymer coatings can be readily sprayed on to the back of sheep and prevent sunburn (Robertson *et al.* 1980) provided they remain attached to the greasy skin (Gordon and Bennett 1980). Australia's sheep industry extends from 20° to 43°S. latitude and spans several climatic zones. Despite this diversity of environment, the risk of sunburning can be predicted for any locality and time of year because the erythemal dose received by a horizontal surface on a cloudless day depends predominantly on solar altitude, i.e. latitude (Robertson 1968; Paltridge and Barton 1978). Solar altitude also determines the anatomical distribution of erythemal radiation over a sheep randomly oriented to the sun (Fig. 2). A convenient threshold for calculating the incidence of significant sunburn in sheep is an erythemal dose of 5 sunburn units which damages the skin to the extent of encrustation,

but does not cause irreparable harm. Using this threshold value in conjunction with Fig. 3, it can be seen that there are marked seasonal and anatomical variations in the risk of sunburning on a clear day at 34°S. latitude. Unprotected, denuded sheep near this latitude would experience substantial sunburn in all months except May, June and July, when exposure on at least 2 consecutive clear days would be required to produce encrusting sunburn. When cloud cover is taken into account, the daily total erythemal dose decreases (Paltridge and Barton 1978), but the risk of sunburning at 34°S. latitude decreases only slightly, and the period of minimal risk would then extend into August. The predictions derived from Fig. 3 would apply to most sheep in Australia since about 80% of the population lies within 4° of 34°S. latitude (Division of National Mapping 1979). However, the average daily total erythemal dose at Cloncurry (20°43′S.) ranges from 10 sunburn units in June to 25 sunburn units in January (Robertson 1972). Thus, sunburn would be a perennial problem, at least on the dorsal surfaces of unprotected, denuded sheep, in the northern wool-growing areas of Australia.

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