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Pigmented Spots in the Wool-Bearing Skin of White Merino Sheep Induced by Ultraviolet Light

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Abstract

Black–grey pigmented skin spots, some of which contained pigmented wool fibres, were observed in a flock of 8·5-year-old white Merino ewes. The spots were concentrated along the backline and increased in number following shearing, suggesting exposure to sunlight to be of importance in the development of these non-congenital pigmented skin spots in genetically white Merino sheep. To test the effect of ultraviolet light, white Merino sheep, ranging in age from 3 to 8 years, had a closely clipped midside area of wool-bearing skin irradiated on each of 28 consecutive days. Pigmented skin spots developed in 6 of the 16 white Merino sheep irradiated. Spots first appeared after 10 days of irradiation, the number subsequently increasing with time, and two skin spots were found to contain sparse numbers of black–grey pigmented wool fibres. Histological examination showed both the naturally occurring and irradiation-induced pigmented skin spots resulted from an increase in both number and activity of melanocytes localized along the epidermal–dermal border of the epidermis. With time, the melanocytes were observed to have entered, to varying depths, the outer-root sheath of follicles still producing white wool fibres. These ultraviolet-light-induced changes to epidermal melanocytes in white Merino sheep presumably occur due to alterations within the local tissue environment in which the melanocytes lie.

Introduction

The amount of melanin present in the hair and wool-bearing skin of mammals determines the level of pigmentation. Melanin is produced within specialized cells called melanocytes in the form of granules, which are then transferred to adjacent cells via the dendritic projections of the melanocytes (Birbeck *et al.* 1956). Deposition of melanin within the skin aids in protecting the underlying tissues from the damaging effects of ultraviolet light (UVL) (Pathak 1967).

In Merino sheep, pigmented wool-bearing skin is normally only seen on black or brown and piebald sheep. Melanocytes within the pigmented wool-bearing skin are present in the epidermis, outer-root sheath and follicle bulb regions (Lyne and Hollis 1968). Black or brown fleece is the result of a recessive gene which follows simple Mendelian inheritance while the piebald phenotype is thought to result from another recessive gene which shows incomplete penetrance (Brooker and Dolling 1969*a*, 1969*b*; Ryder 1980). In white Merino sheep skin the melanocytes are localized at the epidermal–dermal border and are absent from the outer-root sheath and bulb of wool follicles. These melanocytes are sparsely distributed along the epidermal–dermal border and show a low level of melanogenic activity; dendrite projections are poorly developed and melanin was found not to be transferred to adjacent epidermal cells (Forrest *et al.* 1985). In addition, white Merino sheep skin failed to

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tan (uniform darkening of the skin due to increased melanocyte activity) in response to UVL irradiation (Forrest and Fleet 1985).

Field evidence indicates that black skin spots and pigmented wool fibres may develop on genetically white adult Merino sheep (i.e. non-congenital pigmentation). The occurrence of these pigmented spots appears to increase with sheep age (Kelley and Shaw 1942; Fleet and Forrest 1984). Also, the development of pigmented spots was promoted by an additional shearing and it was suggested that exposure of the skin to sunlight following shearing may have been responsible (Fleet and Forrest 1984).

The presence of pigmented fibres in white wool is a concern to the textile industry, especially in the production of white or pastel-shade fabric. The present study was initiated to determine whether the black skin spots seen in the field on aged white Merino sheep could be induced experimentally using an artificial source of UVL. It was of interest also to compare these non-congenital pigmented skin spots with normal white skin from white Merino sheep and pigmented skin from black and piebald Merino sheep.

Methods

Sheep and Location

The sheep were all South Australian strong-wool Merinos located at either Parndana (latitude 35°45·5′) or Turretfield (latitude 34°32′) Research Centres. At Parndana Research Centre in October 1983 a flock of old Merino ewes (aged 8·5 years) was inspected, following shearing, for pigmented skin spots on the dorsal wool-bearing areas and 65% of these ewes were identified as bearing one or more black or grey pigmented skin spots. Five of these sheep were re-shorn 2 months later and observed again.

Samples of pigmented and non-pigmented skin were taken from six sheep at Parndana Research Centre for histological examination. For the purpose of comparison, two skin samples were also taken from two black and two piebald Merino ewes located at Turretfield Research Centre.

Ultraviolet Light Irradiation

At Turretfield Research Centre 16 sheep were used to determine whether black–grey pigmented skin spots could be induced on white sheep skin following exposure to UVL. The sheep were aged 3 years (seven ewes), 5 years (seven ewes) and 8 years (two rams). Three of the 3-year-old sheep were known to be heterozygous (*Ww*) for recessive black fleece. Each sheep had a 30 by 40 cm region along the left midside clipped to a level of approximately 2 mm above the skin. Clipping was repeated twice weekly to ensure wool fibres remained less than 4 mm. The clipped region was irradiated daily for 30 min on 28 consecutive days at a distance of 25 cm from the UVL source (Philips MLU 300 W sun lamp) to the skin. The approximate daily dosage was 18 J/cm^2 of UVL energy (280–400 nm range), and represents only 8·5% of the UVL received daily under natural conditions during January and February. However, the lamp provided 2·2 times the amount of UVL within the 30-min exposure period than the skin would have received under natural conditions. All sheep were inspected daily for the presence of pigmented skin and pigmented wool fibres and again 30 days after the last irradiation. The dimensions of the pigmented spots were recorded and a darkness score allocated using a Stipple reference scale from score 1 (pink), score 2 (very light grey) through to score 8 (black). During the 58-day period the sheep were kept housed, away from direct sunlight. However, after being clipped and inspected on day 58, the sheep were released into a paddock in late autumn and the clipped area received natural sunlight. All sheep, except the two 8-year-old rams, were inspected following their next shearing in spring 135 days later.

Twenty-eight days prior to the final inspection, four of the sheep (Nos 1, 2, 3, 4) which had developed pigmented skin spots by day 58 were housed again and wool clipped from the right midside. These sheep were then subjected to repeated clipping of the wool (twice weekly) for 28 days as had occurred initially for the left midside, but with no irradiation. This additional assessment was undertaken to determine whether clipping alone, by way of minor trauma, could initiate pigmented skin spots. Following inspection

on day 193, two sheep (Nos 2 and 3) were again irradiated daily with UVL, this time the right midside in the same manner as for the initial 28-day period. At this time and at day 193 (left midside) two to four pigmented skin spots per sheep were removed for histological examination.

Histology

Skin samples were collected, fixed, sectioned and stained as previously described (Forrest *et al.* 1985). The number of melanocytes was estimated per square millimetre from horizontal sections through the epidermal–dermal region. These estimates were based on 10 measurements per section from two skin samples for each of the sheep.

Results

Field Observations

Black–grey pigmented skin spots were observed on the dorsal wool-bearing area of 65% of 8·5 year old Merino ewes after close examination following shearing. The spots tended to be concentrated along the backline and upper sides of the sheep. Average spot size and darkness score were estimated to be 7 ± 4 mm diameter and 4 ± 2, respectively. In all, 58% of the skin spots were seen to contain sparse populations of pigmented wool fibres that were rarely recognized during shearing.

Table 1. Numbers of pigmented spots observed on five 8·5-year-old Merino ewes at two shearings 2 months apart

Sheep No.	October shearing		December shearing	
	No. spots with pigmented fibres	Total No. of spots	No. spots with pigmented fibres	Total No. of spots
1	1	2	10	56
2	7	15	17	77
3	30	38	69	123
4	41	55	46	169
5	65	75	119	214
Mean ± s.d.	28·8 ± 26·0	37·0 ± 29·5	52·2 ± 44·2	127·8 ± 65·0

The number of black–grey skin spots on the five sheep re-shorn after 2 months (Table 1) increased by up to 28-fold (paired t-test, $P < 0{\cdot}01$), and numerous brown skin spots, like 'freckles', were also evident at the second shearing. These brown skin spots did not appear to give rise to pigmented fibres and were not seen on sheep shorn at the normal 12-month interval. The increase in the number of pigmented skin spots bearing pigmented fibres was on average about half as great as the increase for the total number of black–grey skin spots. Fig. 1 illustrates one of the 8·5 year old sheep at the first shearing (sheep No. 5, Table 1), showing the black skin spots.

Histology

Non-congenital pigmented spots on white Merino sheep from Parndana showed numerous melanocytes (320 ± 21/mm^2, n = 6) at the epidermal–dermal border, extending to varying depths along the outer-root sheath, and occasionally present in the follicle bulb, resulting in a pigmented fibre (Figs 2 and 3). The melanocytes were darkened, containing numerous active melanosomes, had a highly dendritic appearance and adjacent keratinocytes contained numerous melanin granules.

In contrast, Fig. 4 shows the normal distribution of melanocytes in epidermis of non-pigmented skin (28 ± 9/mm^2, n = 6).

In both black and piebald skin the location and appearance of melanocytes was similar to that described for non-congenital pigmented spots on otherwise white

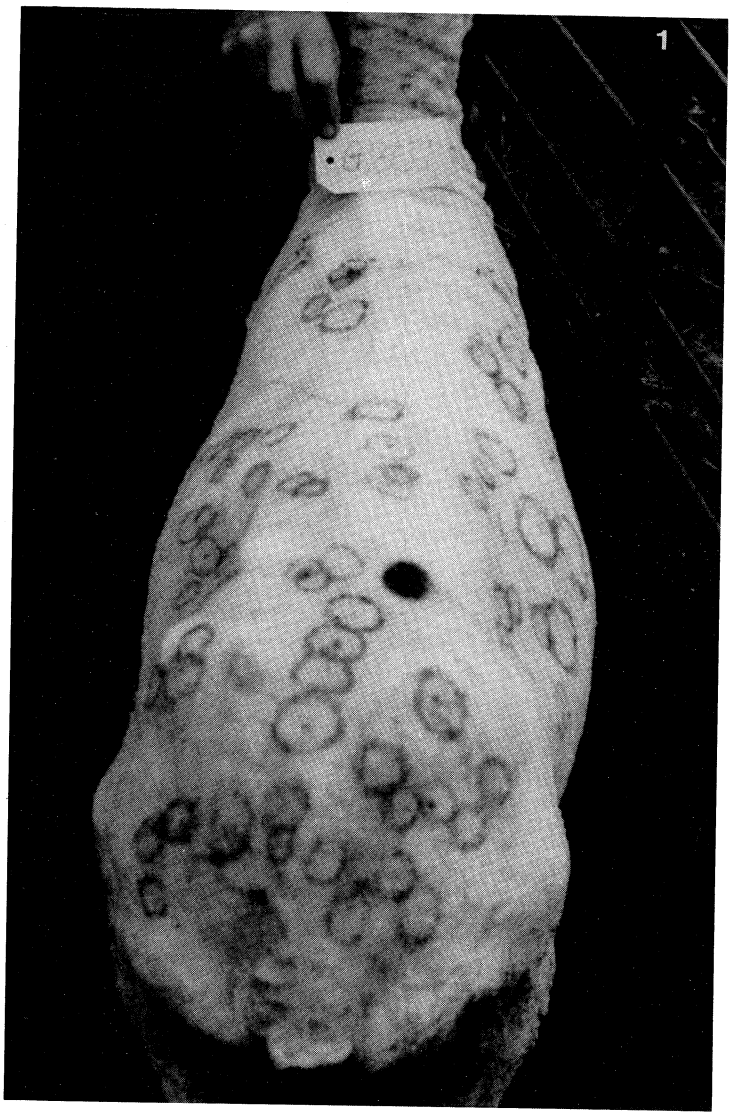
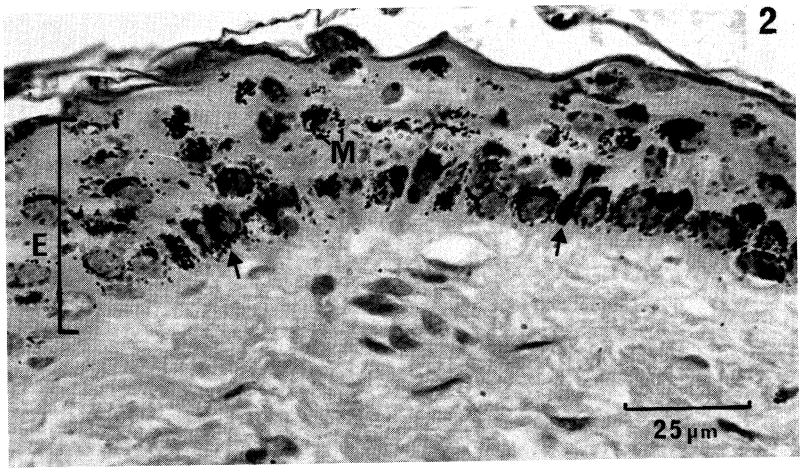
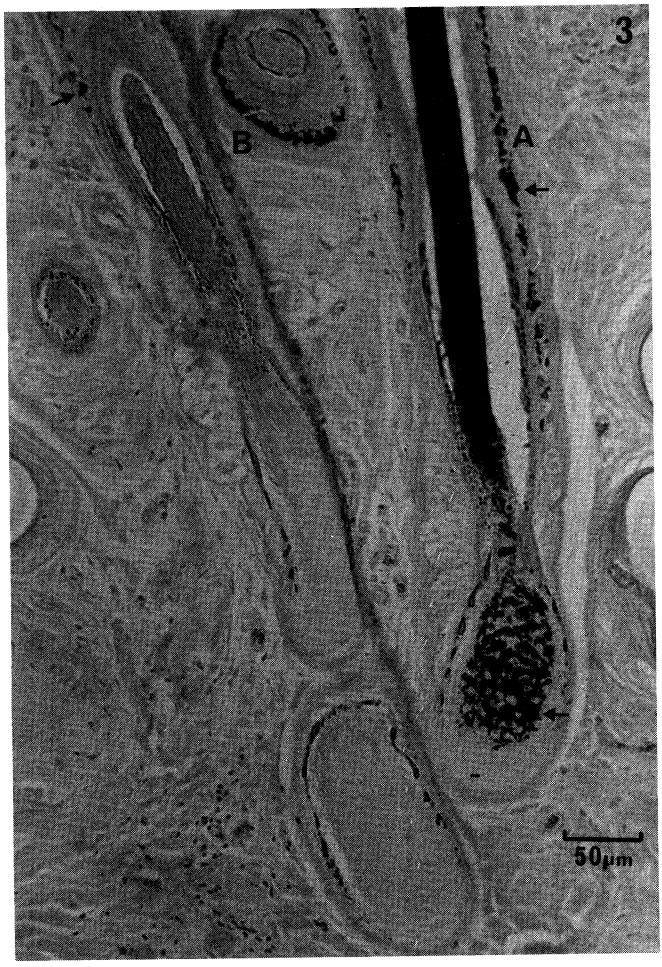


Fig. 1. An 8·5-year-old Merino sheep (Table 1, sheep No. 5) on which 75 black–grey skin spots (encircled in Texta) were observed. The spots were distributed mainly along the back and upper sides of the sheep. Ignore solid mark on centre back of sheep, which is identifying spray mark.

Merino sheep. However, these melanocytes were characteristically unevenly distributed along the epidermis (Fig. 5) and outer-root sheath while the number of melanocytes present in the epidermis (black, 68 ± 11/mm^2; n = 2; piebald, 58 ± 9/mm^2, n = 2) was substantially less than for non-congenital pigmented spots.

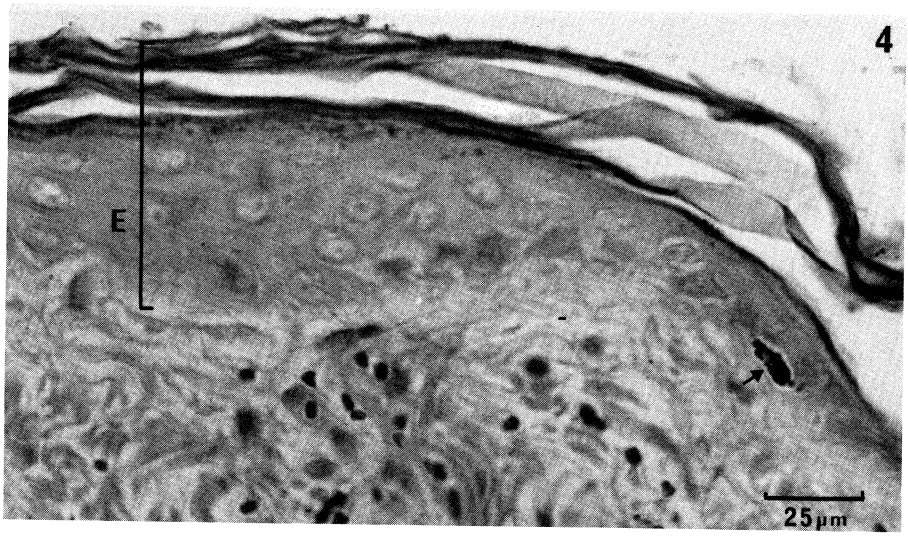


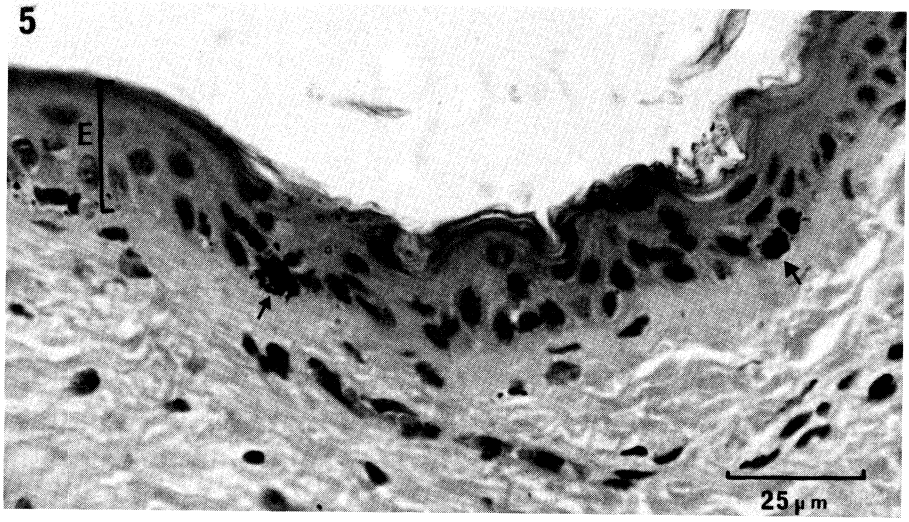


Figs 2 and 3. Vertical sections through a pigmented skin spot of a white Merino sheep. Fig. 2 shows the distribution of melanocytes (arrows) within the epidermis (*E*). Note the large number of melanin granules (*M*) in adjacent keratinocytes. Fig. 3 shows the distribution of melanocytes (arrows) within the bulbs and outer-root sheaths of two follicles. Note follicle A bears a pigmented fibre and has melanocytes along the outer-root sheath and capping the dermal papilla. Follicle B has a white wool fibre but shows evidence of melanocytes in the upper outer-root sheath (arrow).

UVL-induced Pigmented Skin Spots

Daily UVL irradiation ($\simeq 18$ J/cm^2 in 280–400 nm range) on each of 28 consecutive days to the left midside resulted in the development of pigmented skin spots in 6 of the 16 white Merino sheep (two from two of the 8-year-old, three from seven of the 5-year-old and one from seven of the 3-year-old sheep). As Fig. 6 shows, the pigmented spots were distributed randomly across the irradiated skin region.





Figs 4 and 5. Vertical sections through the wool-bearing skin of Merino sheep. Fig. 4, white sheep section showing the presence of a melanocyte (arrow) within the epidermis. Note the thickened epidermis (*E*) due to nine daily dosages of UV irradiation. Fig. 5, black sheep section showing the presence of melanocytes (arrows) within the epidermis (*E*).

As shown in Table 2, spots first appeared on day 10 of irradiation; the number subsequently increasing during the 28-day irradiation period to a maximum of 6. The diameter of the spots on first appearance ranged from 1 to 8 mm and showed a variable increase during the experimental period to a maximum of 12 mm.

Likewise, intensity of pigmentation, adjudged on Stipple scale from 1–8, on first appearance ranged from 3 to 6 and showed only a slight darkening during the experimental period. Four of these six sheep (two 8-year-old and two 5-year-old) also developed light brown skin spots, during the 28-day irradiation period, as did two of the 5-year-old sheep that had not developed pigmented spots.

Table 2. Development of black–grey skin spots after UVL-irradiation of white wool-bearing skin of Merino sheep

For experimental details see Materials and Methods

Sheep No.	Sheep age (years)	Record day	No. of spots	Mean ± s.d. diameter of spots (mm)	Mean ± s.d. darkness score
1[A]	3	14	2	2 ± 1	5 ± 1
		21	4	2 ± 1	6 ± 1
		28	5	2 ± 1	7 ± 1
		58	11	2 ± 1	5 ± 1
		193[C]	11	4 ± 2	4 ± 1
		193[D]	15	3 ± 1	4 ± 1
2	5	10	2	5 ± 4	4 ± 1
		14	3	5 ± 5	5 ± 1
		21	3	5 ± 5	6 ± 0
		28	4	5 ± 5	6 ± 1
		58	7	4 ± 4	5 ± 1
		193[C]	7	6 ± 3	4 ± 1
		193[D]	11	3 ± 2	4 ± 1
3[A]	5	10	1	3 ± 0	3 ± 0
		14	2	2 ± 2	3 ± 1
		21	2	3 ± 3	4 ± 0
		28	6	2 ± 2	4 ± 1
		58	13	2 ± 1	4 ± 1
		193[C]	13	3 ± 2	5 ± 1
		193[D]	36	2 ± 2	4 ± 1
4	5	58	2	2 ± 0	3 ± 0
		193[C]	1	3 ± 0	5 ± 0
5[B]	8	10	1	3 ± 0	3 ± 0
		14	1	5 ± 0	4 ± 0
		21	1	5 ± 0	5 ± 0
		28	1	6 ± 0	6 ± 0
		58	1	6 ± 0	6 ± 0
6[B]	8	14	1	2 ± 0	5 ± 0
		21	1	2 ± 0	4 ± 0
		28	1	3 ± 0	4 ± 0
		58	1	4 ± 0	4 ± 0

[A] The spots recorded were pigmented grey-black and scored for darkness using a stipple reference scale. On day 193 one spot on each of sheep 1 and sheep 3 was recognized as bearing pigmented fibres.

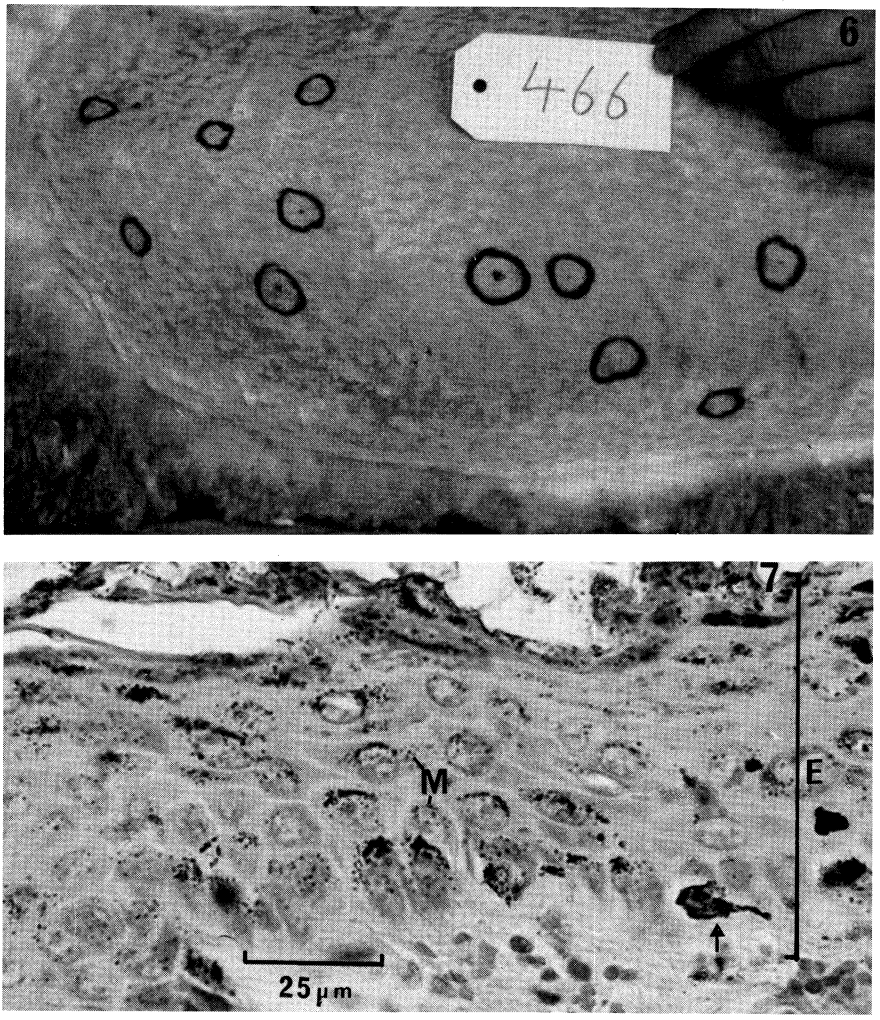
[B] The 8-year-old sheep were excluded from the experiment after day 58.

[C] Results for spots present at day 58.

[D] Results for spots that developed after day 58.

As Table 2 shows, by day 58 the number of pigmented skin spots increased to a maximum of 13 on sheep 3. However, the diameter of spots was not greatly different from that at day 28. Visual observation of the pigmented skin spots failed to reveal

the presence of pigmented fibres. The sheep were then released into the paddock and allowed exposure to sunlight for a further 135 days prior to inspection at spring shearing. No additional sheep developed black–grey skin spots or freckles as a result of exposure to sunlight. In three of four sheep which had developed black–grey pigmented skin spots, the spots present on day 58 were still present on day 193 (Table 2). The spots had increased in diameter but not in intensity of pigmentation.



Figs 6 and 7. UV irradiated skin of white Merino sheep. Fig. 6, left midside of a Merino sheep showing pigmented skin spots (encircled in Texta) 30 days after 28 days of UV irradiation. Fig. 7, vertical section through UVL-induced pigmented skin spot after nine daily doses of UV. Note the presence of a dendritic melanocyte (arrow) within the epidermis (*E*) and the large number of melanin granules (*M*) in adjacent keratinocytes.

These three sheep had developed additional pigmented skin spots, varying in number from 11 to 36, and showed numerous freckles. On each of two sheep one pigmented skin spot was found to contain sparse numbers of black–grey pigmented fibres. The four sheep which had developed pigmented skin spots on their left midside failed

to develop pigmented spots on the right midside as a result of only repeated clipping of the wool. However, when their left midsides were irradiated with UVL pigmented spots developed within 9 days. Histological examination of these pigmented spots (Fig. 7) showed they resulted from an increase in the number and activity of melanocytes localized along the epidermal–dermal border. These melanocytes appeared highly dendritic, darkened by numerous melanosomes and adjacent keratinocytes contained melanin. The number of melanocytes estimated from horizontal sections through the epidermal–dermal border region increased from the control value (21 ± 7/mm^2, n = 4, Forrest and Fleet 1985), to 280 ± 32/mm^2, n = 2. Analysis of day 193 pigmented skin spots revealed the presence of numerous active melanocytes, to varying depths, within the outer-root sheath of otherwise white wool follicles (Fig. 8). The distribution and activity of melanocytes in these sections was comparable to those from the 8·5-year-old sheep (Figs 2 and 3) at Parndana Research Centre.

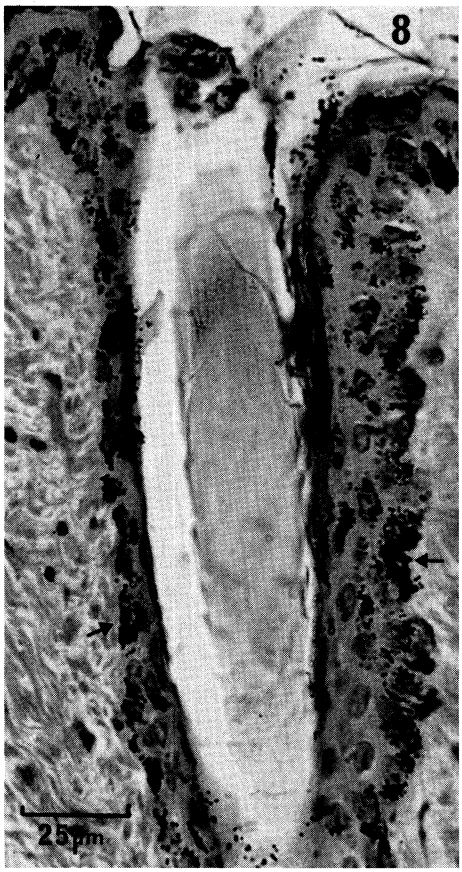


Fig. 8. UV-induced pigmented skin spot of white Merino sheep on day 193. Melanocytes (arrows) are present in the outer root sheath of a follicle growing a white wool fibre.

Discussion

Pigmented skin spots, bearing sparse populations of pigmented wool fibres, were first documented by Kelley and Shaw (1942). This evidence together with more recent observations (Fleet and Forrest 1984) has indicated that old Merino sheep can be prone to developing these spots.

The non-congenital pigmented spots tend to be concentrated along the backline with a dorsoventral gradient, similar to the gradient of incident erythemal ultraviolet radiation estimated for denuded sheep (Chapman *et al.* 1984). Shearing the sheep seemed to promote the development of black–grey skin spots and associated 'freckling' on five 8·5-year-old sheep. These observations are consistent with the hypothesis (Fleet and Forrest 1984) that exposure of the skin to sunlight may be the environmental factor which is triggering this abnormality. In this experiment black–grey pigmented skin spots were induced on 6 of 16 white Merino sheep following repeated exposure of the clipped wool-bearing skin to an artificial source of UVL.

Histological examination of both the natural and experimentally induced pigmented skin spots showed they resulted from the activation and proliferation of epidermal melanocytes and the transfer of melanin granules to adjacent keratinocytes. The increased number of melanocytes contrasted with the sparse distribution in adjacent white skin. Pigmented skin spots first appeared after 9–10 days irradiation. Similar findings have been reported (Quevedo and Smith 1963) for C57BL/St mice, where a noticeable increase in melanocytes was observed after six to eight daily exposures to UVL. On day 193 the black–grey pigmented skin spots showed melanocytes extending as a continuous layer to varying depths down the outer-root sheath of wool follicles. In two pigmented skin spots darkened fibres were observed. The development of pigmented wool follicles parallels the field observations (Fleet and Forest 1984); in both cases it appears that the epidermal melanocytes are able to migrate along the outer-root sheath to the follicle bulb, capping the dermal papilla, from where melanin granules transfer to adjacent keratinocytes in the developing wool fibre. The migratory ability of melanocytes has been proposed as a mechanism whereby pigmented wool-bearing skin develops in non-pigmented regions of Suffolk and black Merino sheep following trauma (Lyne and Hollis 1968) and after skin grafting in grey and white skin patches on black Merino and Soay sheep (Ryder and Priestley 1979; Lyne and Hollis 1980). The requirement of melanocytes to migrate from the epidermis via the outer-root sheath to the follicle bulb, rather than the activation of existing or precursor melanocytes within these regions, agrees with the observed distribution of melanocytes in white Merino sheep (Forrest *et al.* 1985).

The development of black–grey pigmented skin spots in white Merino sheep is one of a number of biological processes influenced by UVL in the skin of mammals. For example, UVL irradiation of mouse skin results in a systemic immunosuppressive effect mediated through a photoreceptor within the stratum corneum (De Fabo and Noonan 1983). UVL can also induce tanning in the skin as a result of the activation and proliferation of melanocytes situated at the junction of the epidermis and dermis (Quevedo and Smith 1963; Quevedo *et al.* 1969; Jimbow *et al.* 1975). However, tanning of the skin in response to UVL has been shown not to occur in white Merino sheep (Forrest and Fleet 1985). UVL can also induce a variety of tumors, both benign and malignant, within the skin (Mackie 1982). Benign tumors like lentigo may lead to lentigo maligna melanoma through malignant change in the epidermal melanocytes. Senile onset lentigo is usually confined to exposed areas of the body. Characteristically the spots appear small, dark brown and flattened due to a limited local proliferation of active melanocytes which may subsequently migrate in the basal layer of the epidermis (Riley 1974). Whilst similarities can be seen between senile onset lentigo in man and UVL-induced pigmented skin

spots in white Merino sheep, none of the experimental animals during or subsequent to UVL irradiation developed malignant melanomas.

How might UVL regulate the activity of melanocytes within the epidermis? Regulation of melanocytes occurs presumably at either the level of the genes encoding melanocytes or the epidermal tissue environment in which the melanocyte lies. Had UVL induced mutations in the gene(s) encoding epidermal melanocytes, then one would have expected to observe, within the skin, characteristic localized hyperactivity of melanocytes with subsequent spread into adjacent dermal areas and the development of malignant melanomas. Alternatively, the cellular environment in which epidermal melanocytes are localized is known in a number of animals to be important in regulating melanocyte activity (Silvers 1979). In white Merino sheep the increased melanogenic activity giving rise to pigmented skin spots may not be a direct response of melanocytes to UVL, but rather an indirect response dependent upon appropriate cues from neighbouring epithelial cells. Possibly a control mechanism within the local tissue environment normally restricts melanocyte activity but becomes less regulated in sheep with increasing age.

Acknowledgments

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