Aust. J. Biol. Sci., 1985, **38**, 245–57

Characterization of Melanocytes in Wool-bearing Skin of Merino Sheep

J. W. Forrest,[A] *M. R. Fleet*[B] *and G. E. Rogers*[A]

[A] Department of Biochemistry, University of Adelaide, Box 498, G.P.O. Adelaide, S.A. 5001.
[B] Department of Agriculture, Animal Industry Division, G.P.O. Box 1671, Adelaide, S.A. 5001.

Abstract

The distribution and character of melanocytes in the wool-bearing skin of Merino sheep of known genotypes were examined by light and electron microscopy. In black Merino sheep (*ww*, homozygous recessive), melanocytes were localized within three regions of the skin: epidermal–dermal border, outer root sheath and follicle bulb. Melanocytes within these regions were found to be actively producing melanin, had numerous dendritic extensions and were able to transfer melanin to adjacent keratinocytes. In a black Merino sheep whose fibres were white due to an experimentally induced copper deficiency the melanocytes were amelanotic. In contrast, for both *WW* (homozygous dominant) and *Ww* (heterozygous) white Merino sheep melanocytes were observed only at the epidermal–dermal border of the epidermis. The melanocytes appeared also to differ in character containing less melanin, appearing less dendritic in shape and having a reduced ability to transfer melanin to adjacent keratinocytes. The gene for white fleece (*W*), therefore, appears able to regulate pigmentation in Merino sheep, at least in part, by controlling the location and activity of melanocytes within the wool-bearing skin.

Introduction

Pigmentation in wool and hair-bearing skin of mammals is due to the presence and activity of the melanin-producing cells (melanocytes). Within the melanocytes melanin is produced in granules (melanosomes) which are then transferred to adjacent keratinocytes via the dendritic projections of the melanocytes (Birbeck *et al.* 1956). Those keratinocytes containing melanin within the follicle bulb are incorporated into the growing wool fibre (Rogers 1979), whilst the melanin in melanocytes and keratinocytes within the epidermis help to protect the skin from the damaging effects of ultraviolet light (Pathak 1967).

The inheritance of pigmented fleece patterns in Merino sheep has been attributed to a single locus (Brooker and Dolling 1969; Ryder 1980). The gene for white fleece (*W*) appears macroscopically to be completely dominant over the genes (*w*) for pigmented fleece patterns (Brooker 1969). Lyne and Hollis (1968) described the distribution of melanocytes in white and black Merino sheep, but did not compare heterozygous (*Ww*) and homozygous (*WW*) white sheep or undertake electron-microscopy studies. White Merino sheep were found to have melanocytes in the epidermis and, on occasions, in upper parts of the follicle. Only in Merino sheep bearing pigmented fibres were melanocytes observed in the outer root sheath and lower bulb region of the follicles. Inactive melanocytes and melanoblasts (precursor melanocytes) would not have been identified by the methods used in their study.

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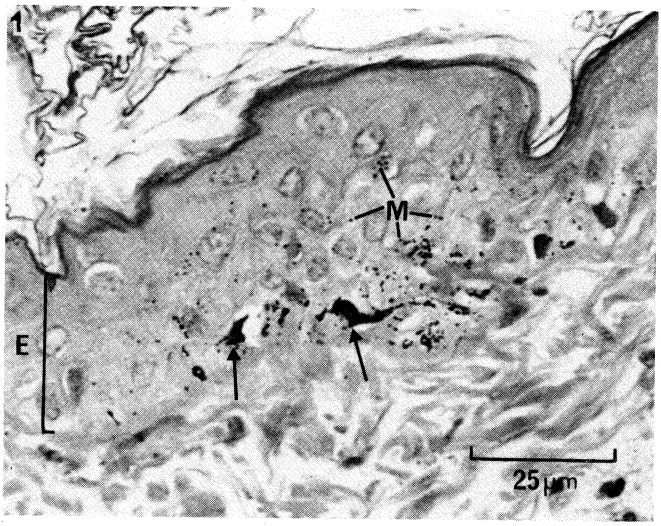
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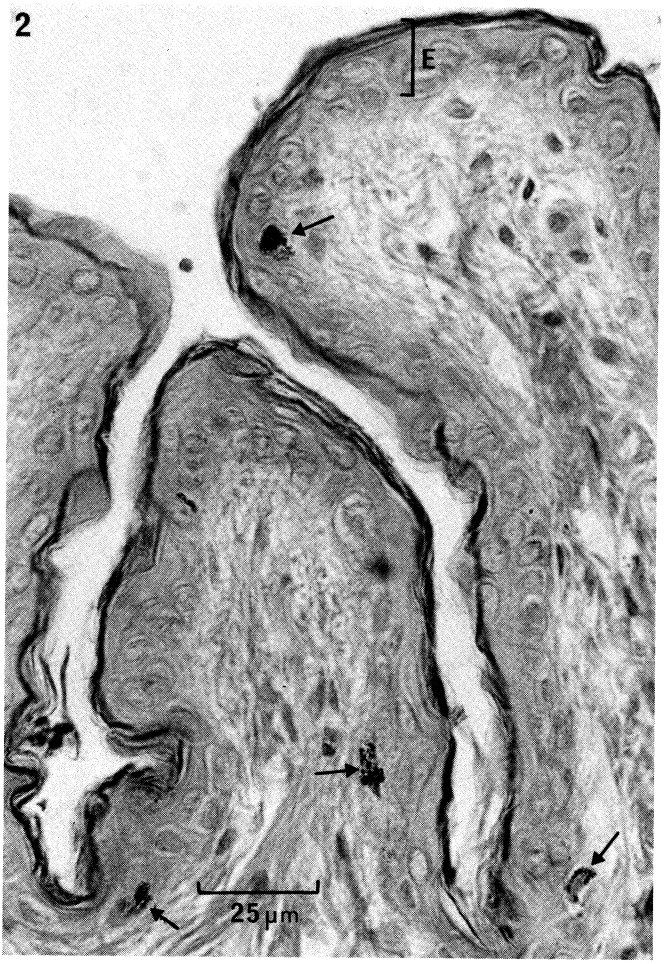
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The aim of the present study was to examine both the distribution and character of melanocytes in the wool-bearing skin of Merino sheep of known genotype. These studies are of particular interest as homozygote and heterozygote white Merino sheep appear phenotypically identical. Knowledge of the distribution and character of melanocytes within wool-bearing skin of Merino sheep may give a clearer understanding of the mechanisms controlling pigmentation.

Methods

Sheep and Location

The sheep were South Australian strong-wool Merinos located at Turretfield Research Centre, South Australia. Three groups of sheep representing the genotypes *WW*, *Ww* and *ww* were used to provide the experimental tissue. The *WW* sheep included two rams (aged 7 years) which were known to be homozygous from the results of matings with black ewes. Ten *WW* ewes (aged 2 years) were progeny of the *WW* rams mated to white (*W*−) ewes. As the gene frequency of *W* in white Merino flocks is low, most of these progeny would have been homozygous (*WW*) for white fleece (Dolling 1979). Ten *Ww* ewes (aged 2 years) were generated from matings between black and white sheep. The six black ewes (*ww*) were 1-year-olds and had the self-colour pattern (Dolling 1979).

Another black Merino ewe (aged 2 years) was made copper-deficient (Dick 1954) by drenching with a daily dosage of ammonium molybdate (60 mg) and sodium sulfate (10 mg). The animal was fed a normal ration of meadow hay but allowed to drink only deionized water. In sheep, copper deficiency results in the loss of fibre pigmentation (Lee and Moule 1947), copper being essential for the activity of tyrosinase, a key enzyme in melanin biosynthesis (Lerner and Fitzpatrick 1950).

Light Microscopy

Skin samples from the midside, taken under local anaesthesia using the trephine method (Nay 1973), were fixed in 10% (v/v) buffered formalin, embedded in paraffin and then sectioned (7 μm) both vertically and horizontally to the skin surface. Sections were then incubated in a solution of L-DOPA (1 mg/ml) in 0·1 M phosphate buffer, pH 7·4, for 4 h at 37°C and/or a 10% (w/v) ammoniacal silver solution for 1 h at 37°C to enhance detection of both active, inactive and precursor melanocytes (Mishima 1960). All sections were then counterstained with haematoxylin and eosin. The number of melanocytes per square millimetre was estimated from horizontal sections through the epidermal–dermal region. These estimates were based on 10 measurements in each of two sections from four sheep of each genotype.

Figs 1–6. Vertical sections through the wool-bearing skin of Merino sheep.

Fig. 1. Black sheep section showing the distribution of melanocytes (arrows) along the epidermal–dermal border of the epidermis (*E*). Note the elongated, dendritic appearance of the melanocytes and the presence of melanin granules (*M*) in adjacent keratinocytes.

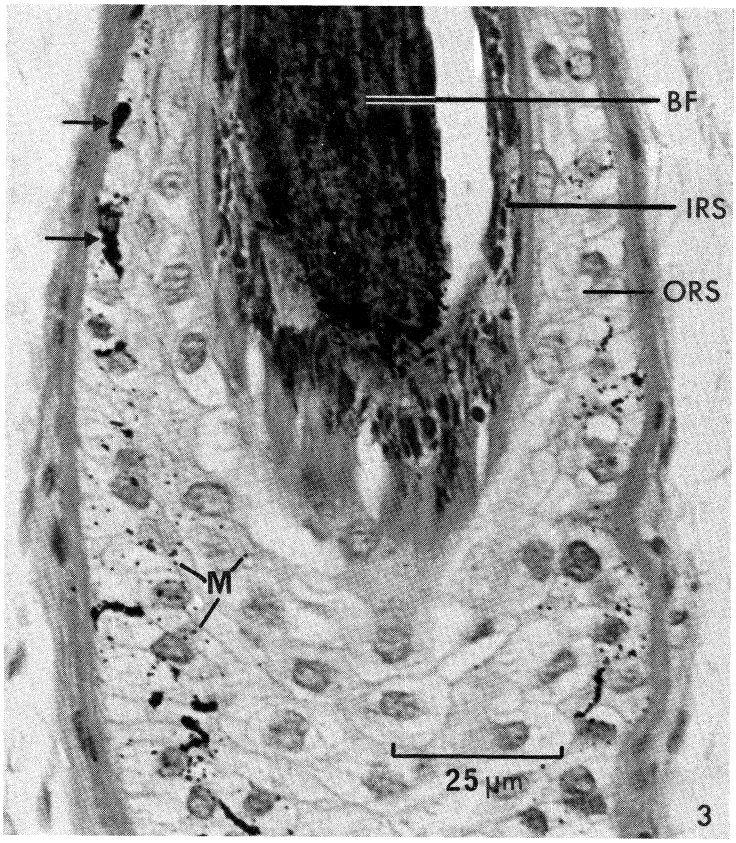
Fig. 2. White (*Ww*) sheep section showing distribution of melanocytes (arrows) along the epidermal–dermal border of the epidermis (*E*). Note the lack of melanocyte dendrites and melanin granules in adjacent keratinocytes.

Fig. 3. Black sheep section showing the distribution of melanocytes (arrows) in the outer root sheath (*ORS*) of the wool fibre. Note the presence of melanin granules (*M*) in adjacent cells. Inner root sheath (*IRS*) containing dark-staining tricohyalin in *IRS*, keratohyalin in epidermis and black fibre (*BF*) are indicated.

Fig. 4. White (*Ww*) sheep section showing white wool fibre (*F*). Note lack of melanocytes and melanin granules in *ORS*.

Fig. 5. Black sheep section showing the distribution of melanocytes (arrows) capping the dermal papilla (*DP*) in the follicle bulb.

Fig. 6. White (*Ww*) sheep section showing follicle bulb region. Note lack of melanocytes adjacent to *DP* and absence of melanin granules in keratinocytes (*K*). All sections incubated in L-DOPA and ammoniacal silver and counterstained with haematoxylin and eosin.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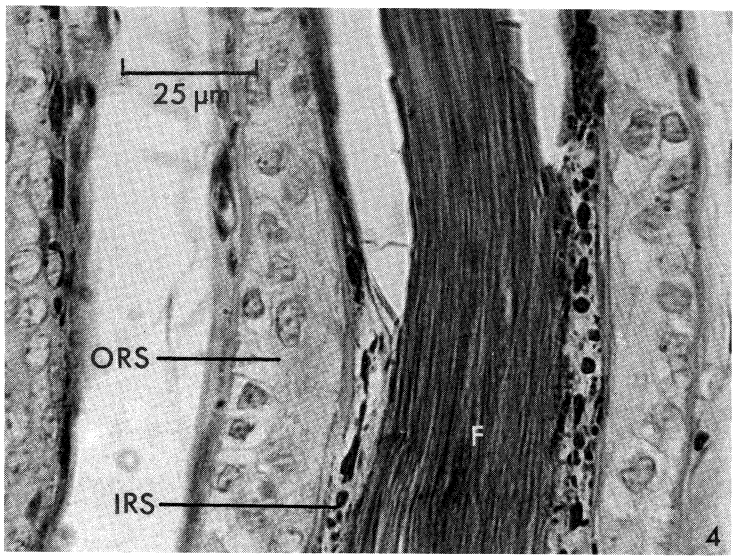
3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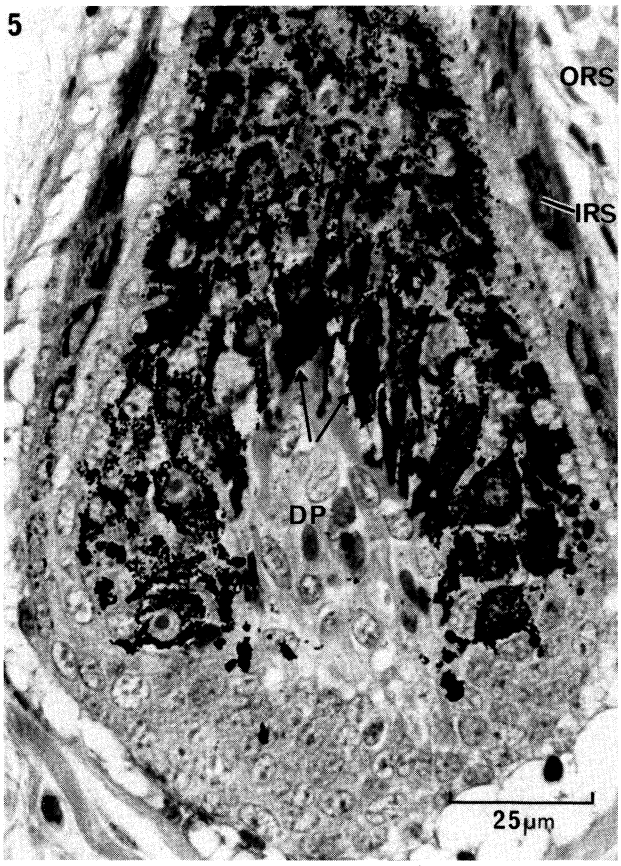
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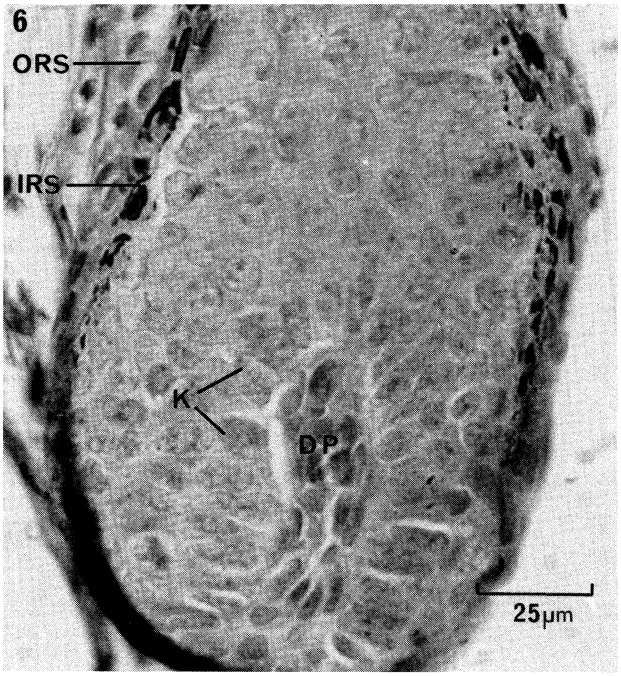
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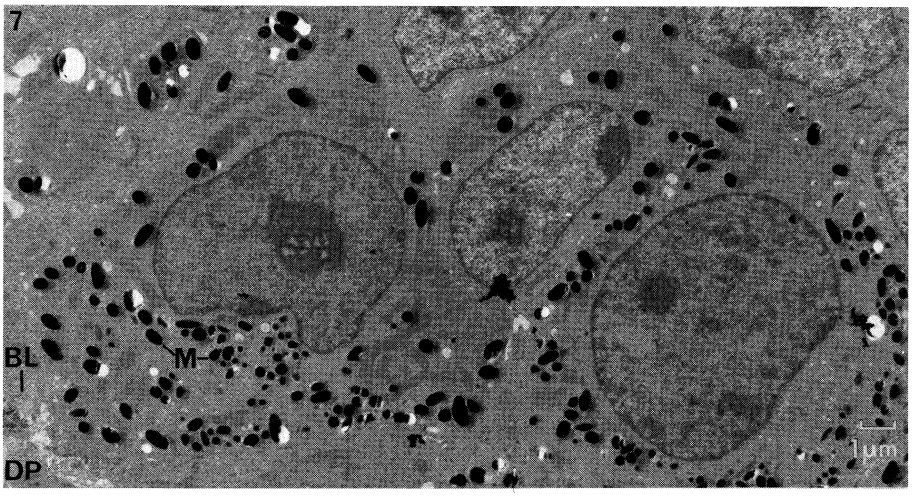
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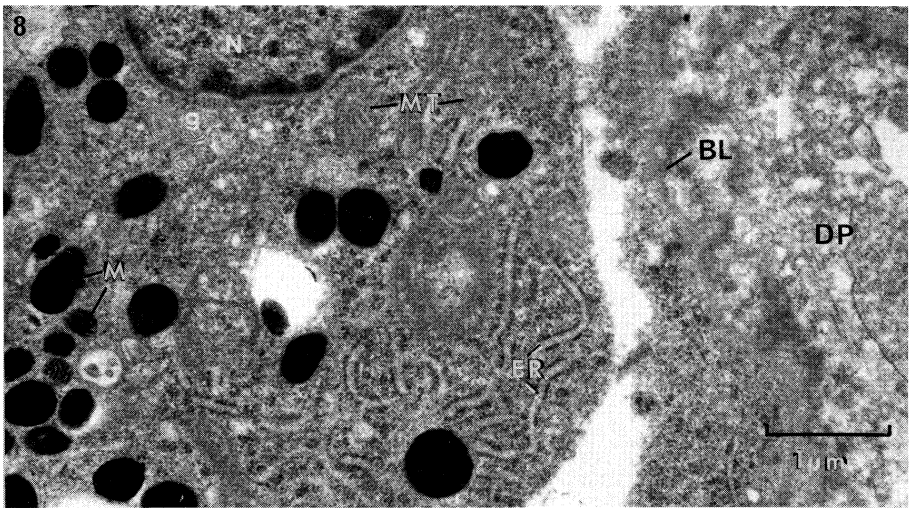
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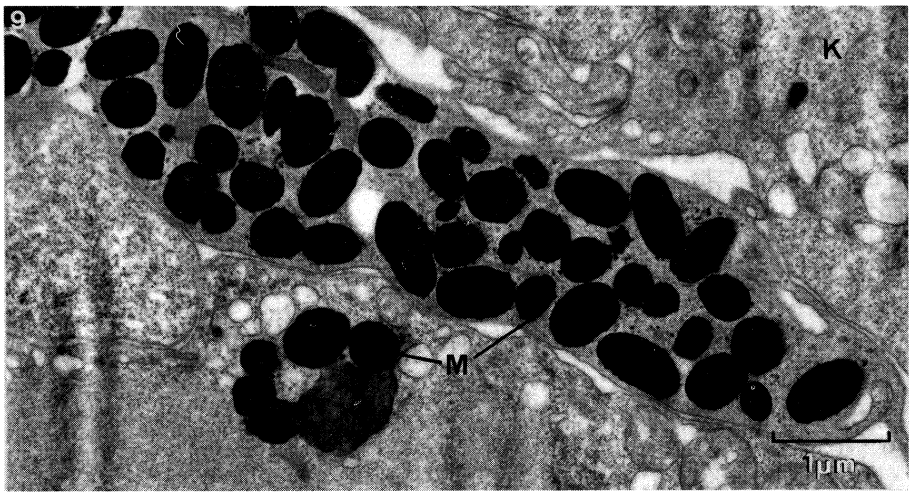
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Electron Microscopy

For electron microscopy, individual wool follicles dissected from skin samples were fixed in 1·5% (v/v) buffered glutaraldehyde–formaldehyde, post-fixed in 1% (w/v) osmium tetroxide, dehydrated through alcohol solutions and embedded in Spurr's resin. To enhance the detection of both active and inactive or precursor melanocytes some follicles were also incubated, following the initial fixation, in L-DOPA and/or ammoniacal silver (Mishima 1964). Thin sections were stained in Reynolds lead citrate followed by a saturated 70% (v/v) ethanolic solution of uranyl acetate and examined using a Seimens 102 electron microscope.

Results

Light Microscopy

Examination of black wool-bearing skin showed the presence of melanocytes in the epidermis, outer root sheath (ORS) and follicle bulb (Figs 1, 3 and 5). In the epidermis the melanocytes appeared localized at the epidermal–dermal border region but varied widely in frequency ($56 \pm 18/mm^2$). They were dendritic and appeared to distribute melanin granules to adjacent keratinocytes (Fig. 1). Dermal melanocytes were infrequent, being most prevalent near the follicles, adjacent to sweat gland ducts and sebaceous glands. Melanocytes were also present among the peripheral cells of the ORS and, as in the epidermis, were variable in concentration (Fig. 3). Within the follicle bulb, melanocytes were usually present in the zone of proliferating cells as a dense population capping the dermal papilla (Fig. 5).

The distribution of melanocytes in the wool-bearing skin of white *WW* Merino sheep was similar to that in the skin of the white *Ww* sheep. As Fig. 2 shows, melanocytes are localized at the epidermal–dermal border region occasionally extending into the ORS to approximately the level of the sebaceous glands. No melanocytes were observed in either the ORS below the sebaceous glands (Fig. 4) or capping the dermal papilla in the follicle bulb (Fig. 6). The melanocytes present in the epidermis varied in concentration ($24 \pm 9/mm^2$), were less frequent than in the black sheep and had a low level of activity, whilst adjacent keratinocytes appeared to lack melanin (Fig. 2).

Electron-microscopic Characterization

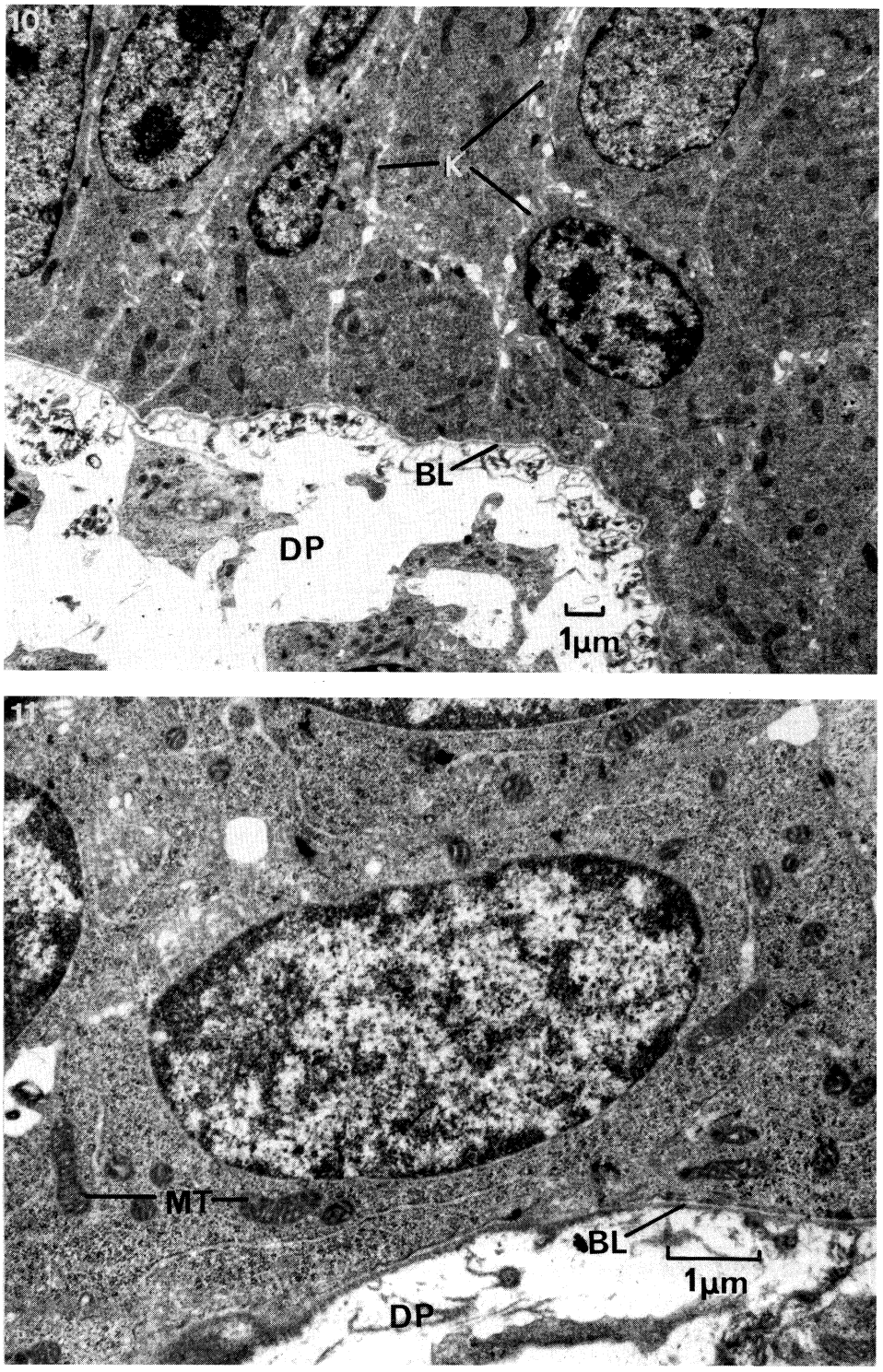
Examination of follicle bulb region of black wool fibres confirmed the presence of melanocytes within the zone of proliferating cells. As shown in Fig. 7, melanocytes were localized close to the basal lamina, capping the dermal papilla, and were adjacent to keratinocytes. They were distinguished from keratinocytes by their larger more dendritic form and presence of numerous melanosomes (Figs 8 and 9). Also, within the cytoplasm they contained a region of well-developed, rough endoplasmic reticulum and golgi apparatus from which melanosomes are synthesized (Fig. 8).

Figs 7–11. Electron micrographs of section wool follicle bulbs from Merino sheep. Figs 7, 8 and 9 are black sheep sections showing melanocyte(s) adjacent to the basal lamina (*BL*) capping the dermal papilla (*DP*).

Fig. 7. Note the large number of active melanosomes (*M*) within the cytoplasm of the melanocytes.

Fig. 8. Cytoplasm of the melanocyte showing melanosomes (*M*), endoplasmic reticulum (*ER*), golgi (*g*), mitochondria (*MT*) as well as prominent nucleus (*N*).

Fig. 9. Dendritic arm of a melanocyte containing numerous melanosomes adjacent to a keratinocyte (*K*).



Figs 10 and 11. White (*Ww*) sheep sections showing keratinocytes (*K*) adjacent to the basal lamina (*BL*) capping the dermal papilla (*DP*). Mitochondria (*MT*) can be seen within the cytoplasm of the keratinocytes. Note the absence of melanocytes or precursor structures.

Serial sectioning through the follicle bulb region of both *Ww* and *WW* white wool fibres, incubated in L-DOPA and ammoniacal silver solutions, failed to reveal the presence of active or inactive melanocytes (Figs 10 and 11). Neither were precursor melanocytes, characterized by poorly developed cytoplasmic processes and large oval nuclei, observed. In all follicles examined, only keratinocytes were localized close to the basal laminae capping the dermal papillae.

Induced copper deficiency in a black Merino sheep resulted in wool-bearing skin similar in colour to a white Merino sheep. Examination of wool follicles revealed the presence of amelanotic melanocytes within the zone of proliferating cells capping the dermal papilla (Fig. 12). These cells were identical to active melanocytes with the exception that their melanosomes lacked melanin (Fig. 13). Prior incubation of the follicles with L-DOPA did not alter the appearance of the melanocytes. However, incubation in ammoniacal silver solution darkened the amelanotic melanosomes within the cytoplasm of the melanocytes (Figs 14 and 15).

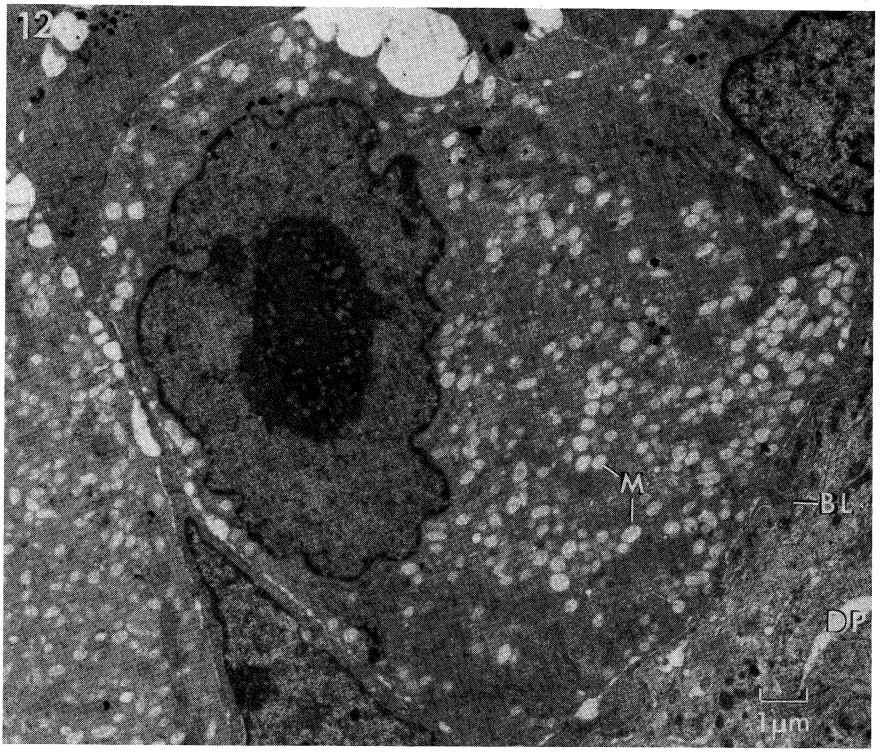
Discussion

The present light-microscopic study has delineated the distribution of melanocytes in the wool-bearing skin of Merino sheep of different genotypes for fleece pigmentation. In general, the distribution of melanocytes was similar to that found in a previous study on pigmentation in sheep (Lyne and Hollis 1968). Use of electron-microscopic cytochemistry on wool follicles has, in addition, allowed the structural characterization of melanocytes within the wool follicle.

For black Merino sheep (*ww*), active melanocytes are present in three distinct skin regions: the epidermal–dermal border of the epidermis, ORS and follicle bulb. Occasionally melanocytes were present within the dermis, although the numbers appeared less frequent than previously reported (Lyne and Hollis 1968). In common with other mammals (Ortonne and Benedetto 1982) melanocytes within the follicle bulb were found localized adjacent to the basal lamina capping the dermal papilla. The melanocytes contained numerous melanosomes (concentrated within their dendritic arms—Fig. 9), which were able to be transferred to adjacent keratinocytes and hence be incorporated into the developing wool fibre (Birbeck *et al.* 1956).

White Merino sheep, both homozygote (*WW*) and heterozygote (*Ww*), had melanocytes only within one region: the epidermal–dermal border of the epidermis. While the numbers of melanocytes were only slightly lower than in the epidermis of black sheep, they had a lower level of melanogenic activity, were less dendritic and appeared not able to transfer melanin to adjacent keratinocytes. Similar findings have been reported for yellow mice and in mice homozygous for the gene '*p*' (pink-eye), where epidermal melanocytes have a low or variable level of melanogenic activity and, in common with white Merino sheep (*WW* and *Ww*) (Forrest and Fleet 1985), failed to tan in response to ultraviolet light irradiation (Quevedo and Smith 1963). In another strain of mice, homozygous for the gene '*d*' (dilute), a reduced tanning response (weaker uniform darkening of the skin following exposure to ultraviolet light) was attributed to melanocytes having fewer dendrites, probably due to inhibitors within the tissue environment (Quevedo and Smith 1963).

Histological examination of wool-bearing skin in white Merino sheep failed to reveal melanocytes within the ORS or follicle bulbs. Incubation of the sections with L-DOPA and/or ammoniacal silver solutions, known to enhance the detection of active and inactive melanocytes (Mishima 1960), also failed to reveal the presence of melanocytes.


12
M
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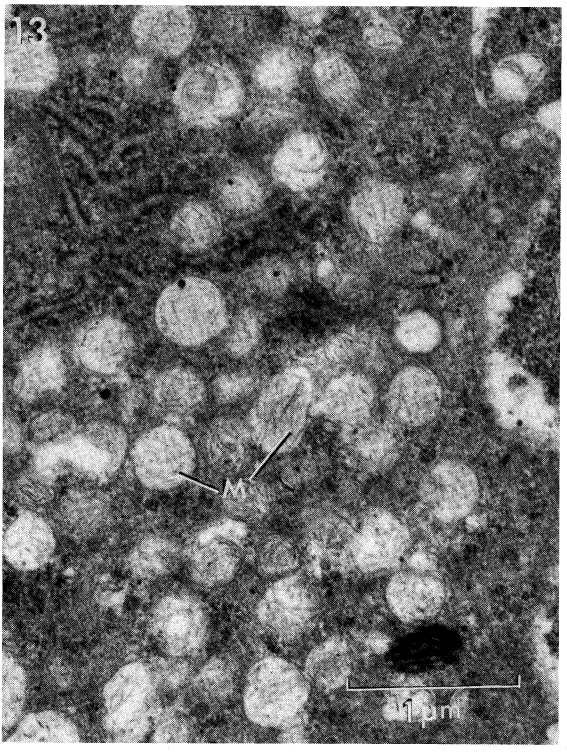
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However, recognizing the presence of melanocytes with light microscopy requires a certain minimal level of melanogenic activity. In addition, as reported for mice (Jimbow and Uesugi 1982), the possibility exists that there are precursor melanocytes which are both enzymatically and structurally inactive. Electron microscopy was used to examine these possibilities in homozygote and heterozygote white wool fibres. As shown in Figs 10 and 11, serial sectioning through the follicle bulb region did not reveal either melanocytes with a low level of melanogenic activity or precursor melanocytes (Jimbow and Uesugi 1982). The inability to detect either form of melanocyte appears not to be a result of the electron-microscopic procedure, since amelanotic melanocytes were readily discernible in a genotypically black Merino whose wool fibres were white through induced copper deficiency.

Knowledge of the distribution of melanocytes in the skin of Merino sheep of known genotypes gives an insight as to how pigmentation may be controlled. The absence of melanocytes or their precursors in white Merino wool fibres suggests that regulation is not the result of an inability of existing melanocytes to synthesize melanin, as for example in albinoism (Russell and Russell 1948). However, the findings from skin-grafting experiments (Ryder and Priestley 1979), the occurrence of isolated pigmented wool fibres (Fleet *et al.* 1984) and the development of pigmented spots in 8-year-old white Merino sheep (Fleet and Forrest 1984) shows that even in genetically white sheep the location and activity of melanocytes in the skin can change in some circumstances.

The location and activity of melanocytes within the wool- and hair-bearing skin of mammals are regulated by the major genes controlling skin and coat coloration (Quevedo and Fleishman 1980). The distribution of melanocytes within the skin is usually determined during embryonic development (Silvers 1979). The absence of melanocytes from wool follicles of white sheep may reflect an embryonic difference which prevents the incorporation of melanocytes into developing wool fibres.

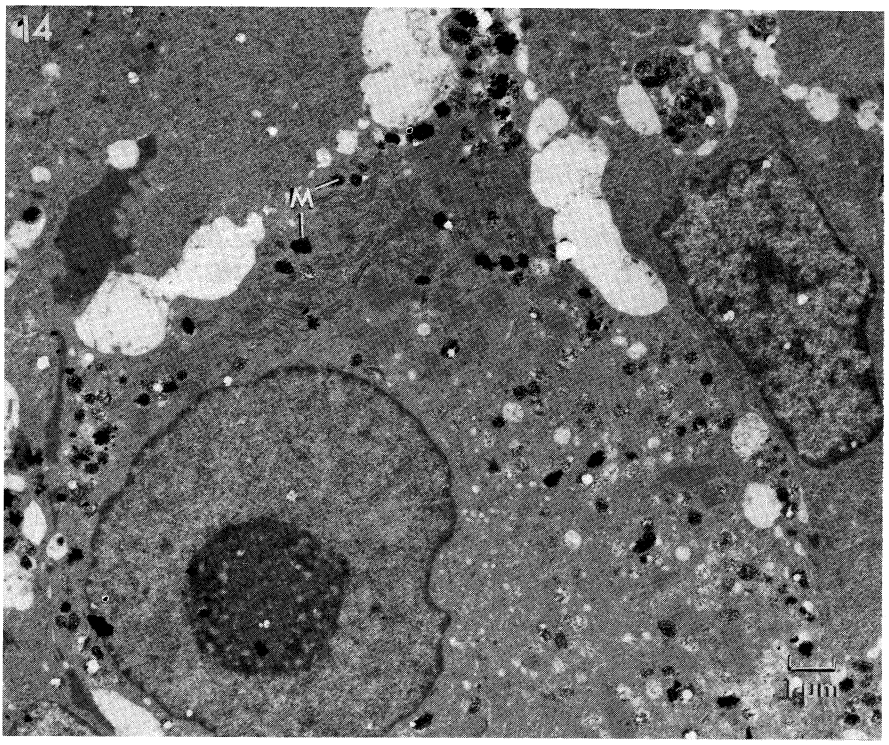
Regulation of existing melanocytes occurs presumably at either the level of the genes encoding melanocytes or the tissue environment in which the melanocytes lie. In the wool-bearing skin of white Merino sheep, a possible control mechanism in the local tissue environment is suggested by the fact that although melanocytes are absent from the wool follicle they are present in the epidermis and have the potential to change their level of expression (Fleet and Forrest 1984; Lyne and Hollis 1980). Further studies are needed in order to determine the influence of the specific components controlling the distribution and activity of melanocytes in white Merino sheep.

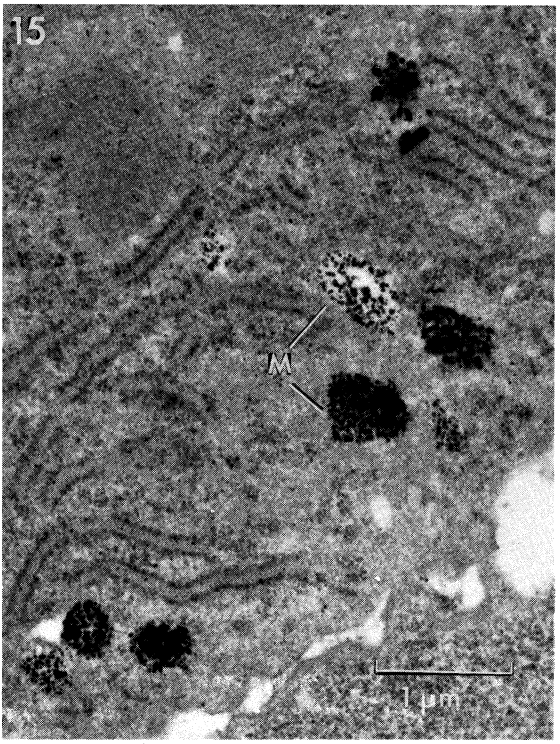
Acknowledgments

We wish to thank Elaine Batty, Michael Calder, Lesley Crocker and Chris Leigh for assistance in the preparation of samples for light and electron microscopy. This work was supported by the Wool Research Trust Fund on the recommendation of the Australian Wool Corporation.

Figs 12–15. Electron micrographs of sectioned wool follicle bulbs from a black Merino sheep rendered copper-deficient to induce loss of pigmentation within the wool fibre.

Figs 12 and 13. Amelanotic melanocytes adjacent to the basal lamina (*BL*) and the dermal papilla (*DP*). Note the large number of melanosomes (*M*) lacking melanin within the cytoplasm of the melanocyte.





Figs 14 and 15. Incubation of the wool follicles in the presence of an ammoniacal silver solution resulted in the deposition of silver within the melanosomes (*M*) in the melanocytes.

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Manuscript received 10 December 1984, accepted 29 July 1985