THE MELANOCYTE POPULATION IN THE SKIN DURING DEVELOPMENT OF THE MARSUPIAL *TRICHOSURUS VULPECULA*

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Summary

Changes in the melanocyte population have been examined in 170 samples of skin from 41 animals, ranging in age from embryos to adults. The earliest melanocytes in samples of epidermis and dermis incubated in DOPA reagent are revealed at 2 days after birth. Melanocytes in the epidermis are present only during the period of follicle initiation from the epidermis, that is, from 2 to 100 days after birth. Dermal melanocytes are confined to the mid-level of the dermis, except in very young animals which have some near the epidermis. Melanocyte numbers in whole mounts of skin range from 0 to 570 per 1 mm^2 in the epidermis and from 0 to 100 per 1 mm^2 in the dermis. Some of the epidermal melanocytes discharge melanin granules into neighbouring epidermal cells. Dermal melanocytes do not release their granules.

Those hair follicles which develop directly from the epidermis carry down epidermal melanocytes. A few melanocytes might also enter the bulbs of these follicles from the dermis. Subsequently, at a stage when there are no melanotic melanocytes in the epidermis or in the outer wall of the follicles, many additional follicles develop by branching from previously formed follicles. Melanocytes in these branched follicles are first seen in the bulb region at the hair cone stage of development, suggesting either that these follicles carry down a complement of precursor melanocytes or that the latter may enter from the dermis.

I. INTRODUCTION

The brush-tailed possum, *Trichosurus vulpecula*, widely distributed in Australia, is one of the most studied of the marsupials. Even so, the skin and its pigmentary system have received little attention. Gibbs (1938) mentioned pigmentation in developing hair follicles in this species but made no reference to the epidermal and dermal melanocytes. Bolliger and Hardy (1945) also studied this marsupial and noted the pigmentation of the skin of advanced pouch young and the unpigmented epidermis of the adult.

There are considerable advantages in using marsupials in preference to eutherian (higher) mammals for such studies as body growth and hair follicle development (Lyne 1957; Lyne and Verhagen 1957). The young is very immature at birth and develops in a pouch where it is accessible for observation and experiment. In *T. vulpecula* the gestation period is 17–18 days and the young at birth is about 13 mm long and weighs 0·2 g (Lyne, Pilton, and Sharman 1959). The adult (more than 1 yr old) is about the size of a domestic cat.

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In the present study, changes in the melanocyte population have been examined in the skin of animals ranging in age from embryos to adults; this information on melanocytes is necessary for more detailed studies of the skin and hair follicles under experimental conditions.

II. Material and Methods

(a) *Animals*

Forty-one animals (2 embryos at 15 and 18 days after mating, 3 new-born young, and 36 which were pouch young when first sampled) were used in this study. Twenty-six of the pouch young were born in captivity. Approximately equal numbers of males and females were used. Ages of pouch young for which birth dates were unknown were estimated by comparison of some body measurements with those of animals of known age (Lyne and Verhagen 1957).

(b) *Skin Sampling and Methods*

One hundred and seventy skin samples from the dorsal or lateral aspects of the body were obtained from the 41 animals. A trephine (1·0 or 0·5 cm in diameter) or scissors were used for sampling; 16 animals were sampled more than once. The number of samples taken before or at birth was 5; birth to 30 days, 34; 31–60 days, 31; 61–90 days, 23; 91–120 days, 17; 121–180 days, 24; 181–365 days, 23; 1–7 years, 13. Whenever possible, samples were divided into two or more parts.

Samples for general histological studies were fixed in Zenker's fluid, Bouin's fluid, or 10% formalin. The DOPA (L-3,4-dihydroxyphenylalanine) reaction, which demonstrates melanocytes, was carried out using the method of Quevedo *et al.* (1966). Samples taken for electron microscopy were fixed in OsO_4. Most other histological and histochemical techniques used were similar to those described by Lyne and Hollis (1968). Some sections were cut perpendicular and others parallel to the skin surface.

Because of the practical difficulties of counting melanocytes in all samples, counts were made only on some samples and a visual system of estimation was devised, whereby the melanocytes in the epidermis or dermis were classified as absent, sparse, common, or dense. These classes usually represented numbers of melanocytes per 1 mm^2 as follows: sparse, 1–50; common, 51–400; dense, more than 400. The latter class was not observed in the dermis.

III. Observations

(a) *Epidermal and Dermal Melanocytes*

The occurrence of melanocytes in the epidermis and dermis of samples of various ages up to 161 days after birth, is indicated in Figure 1. The skin samples from the embryos and new-born young lack recognizable melanocytes but the DOPA reagent was not available when these early samples were taken. The earliest melanocytes in the epidermis and dermis are revealed 2 days after birth in DOPA-incubated samples from two animals. The melanocytes in the epidermis, which are weakly DOPA-reactive and contain a moderate number of melanin granules in both of these animals, are dendritic and common in one (Fig. 2) and mostly rounded and sparse in the other. At this age, no melanin granules are revealed in the adjacent epithelial cells. The DOPA-reactive dermal melanocytes in both 2-day-old animals are rounded or have short dendrites (Figs. 3*A* and 3*B*). Samples not incubated in DOPA reagent do not reveal melanocytes in these animals. In animals older than 2 days, the numbers of melanocytes in samples incubated in DOPA reagent do not appear to differ from those in untreated samples.

The numbers of melanocytes estimated in whole mounts of skin from pouch young less than 100 days old range from 0 to 570 per 1 mm^2 in the epidermis and up to 100 per 1 mm^2 in the dermis. Figure 4 shows a whole mount preparation of skin with a dense population of epidermal melanocytes (approximately 450 per 1 mm^2). This sample was taken 43 days after birth. Epidermal melanocytes (Fig. 1) are most abundant from about 30 to 50 days after birth; they are less numerous but common from 50 to 80 days. Samples taken from 80 to 100 days have only a sparse population of epidermal melanocytes and those taken after 100 days are without recognizable melanocytes.

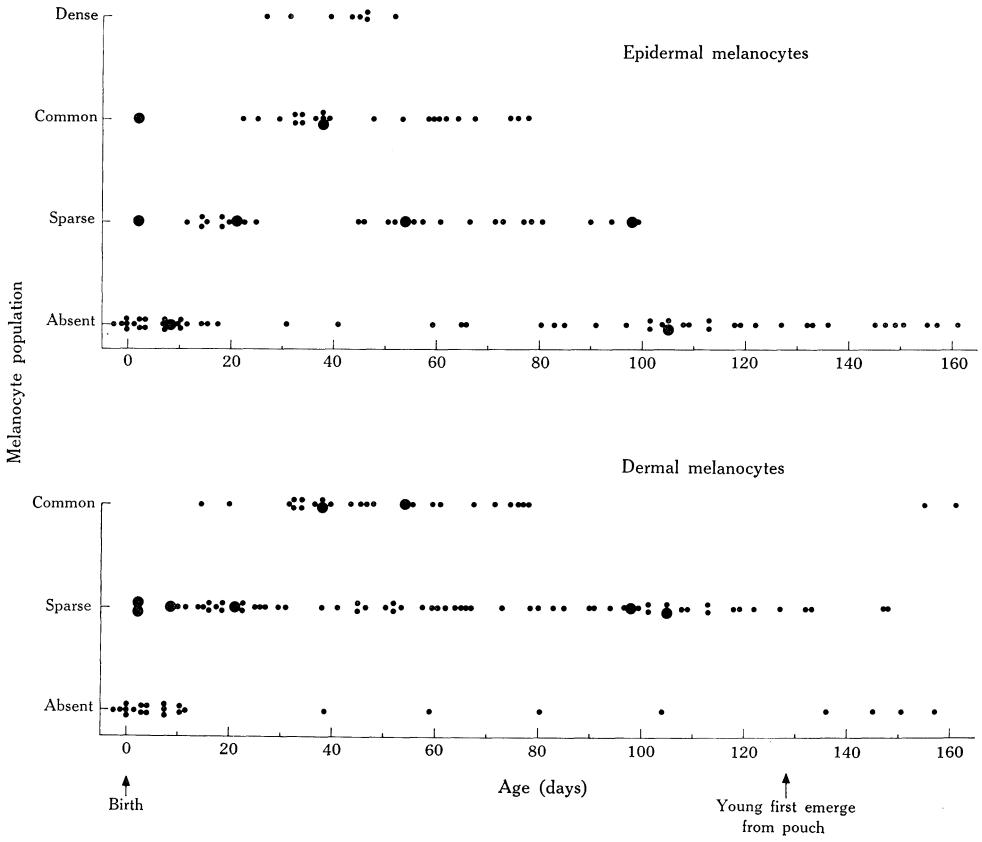


Fig. 1.—Classification of epidermal and dermal melanocytes in 115 samples of various ages. Small dots: samples not incubated in DOPA reagent; large dots: samples incubated in DOPA reagent.

Most epidermal melanocytes are located between the cells of the basal layer of the epidermis (Figs. 5 and 12). The rare occurrence of an epidermal melanocyte above the basal layer (Fig. 5) suggests that very few melanocytes move out to the skin surface to be shed with the cornified epidermal cells. When melanocytes are common or dense in the basal layer, at least some of them discharge melanin granules into neighbouring epidermal cells (Fig. 6). Figure 7 shows the melanin granules in a typical epidermal melanocyte.

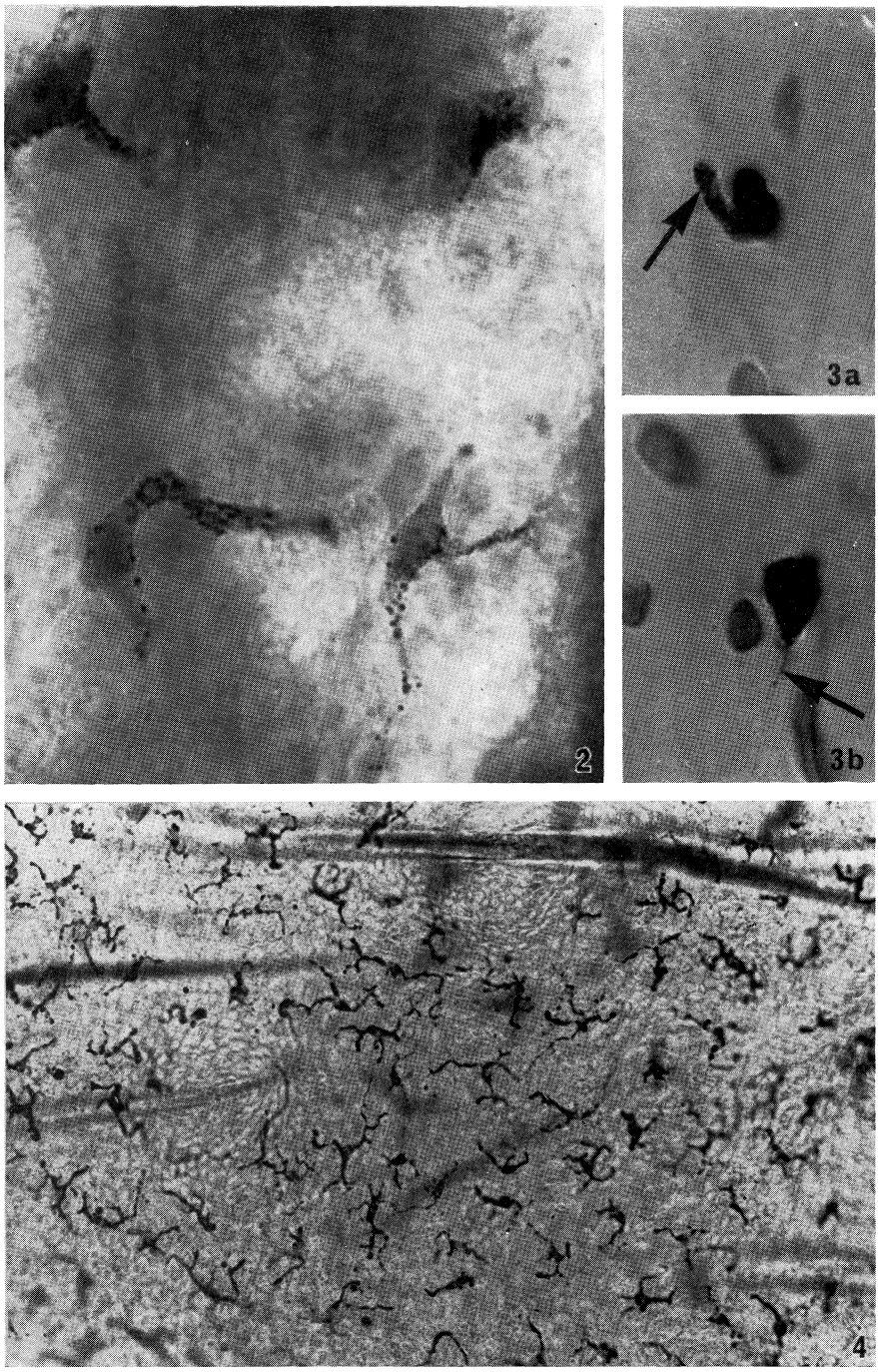
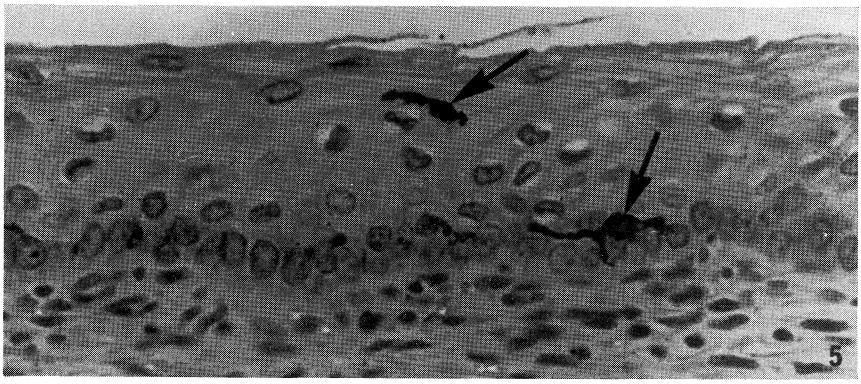
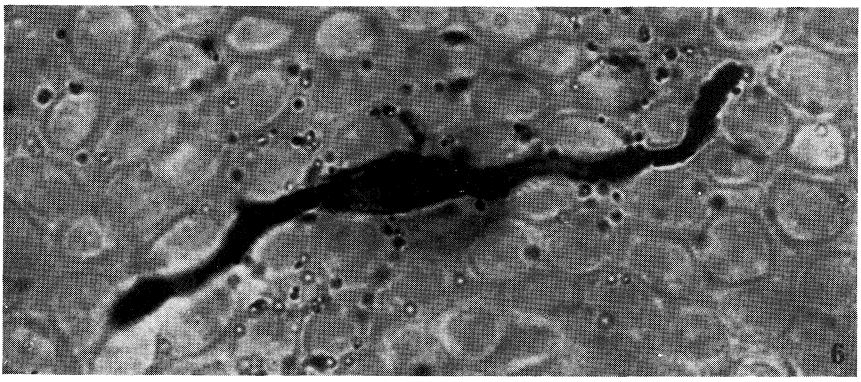


Fig. 2.—Whole mount preparation of skin from a 2-day-old animal. The dendritic epidermal melanocytes are weakly DOPA-reactive with a moderate number of melanin granules. ×1,400.

Figs. 3*a* and 3*b*.—Isolated DOPA-reactive dermal melanocytes in the skin of a 2-day-old animal. Arrow indicates short dendrite of each melanocyte. ×1,400.

Fig. 4.—Whole mount preparation of skin from a 43-day-old animal with a dense population of epidermal melanocytes. Safranin. ×220.





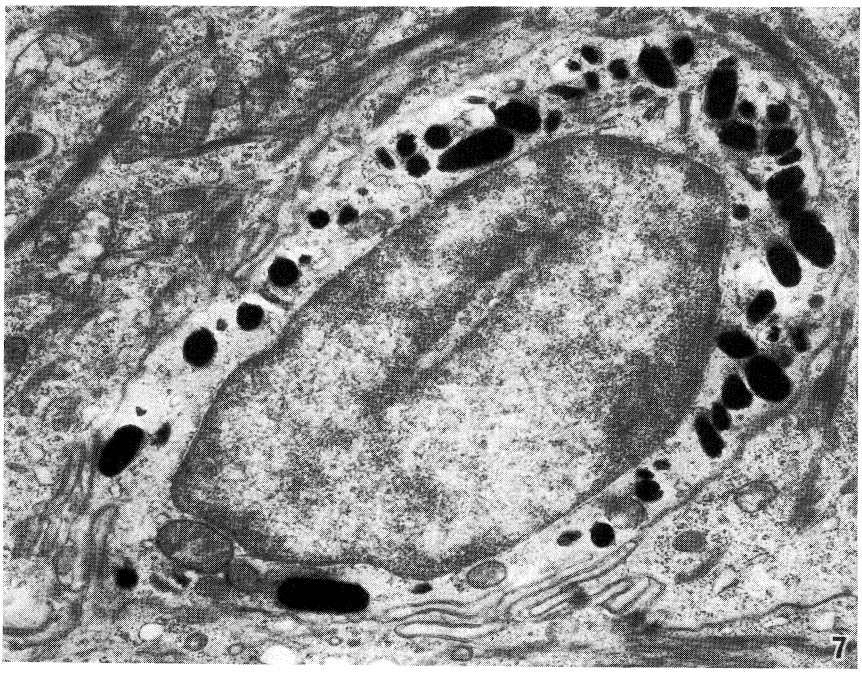
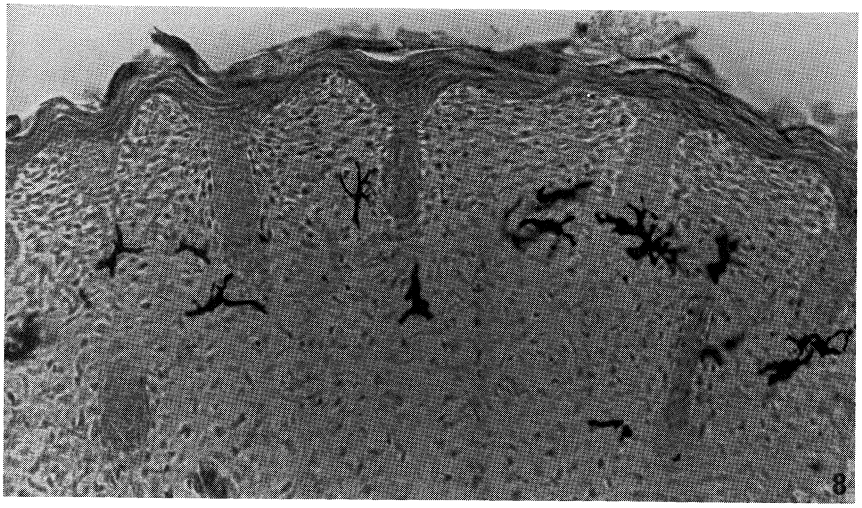
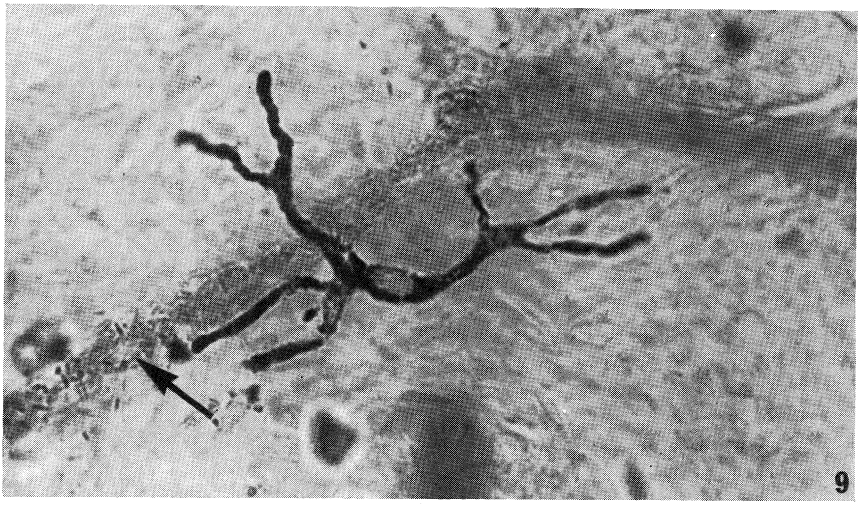


Fig. 5.—Vertical section of epidermis adjacent to sample shown in Figure 4 and at the same age. Note the melanocyte in the basal layer (lower arrow) and another in the upper part of the epidermis (upper arrow). Haematoxylin and eosin. ×560. Fig. 6.—Whole mount preparation of skin from a 46-day-old animal showing a melanocyte at the level of focus of the basal layer of the epidermis and melanin granules in adjacent epidermal cells. Carmine. ×1,400. Fig. 7.—Electron micrograph of a melanocyte in the basal layer of the epidermis of a 55-day-old animal. Uranyl acetate and lead citrate. ×22,570.





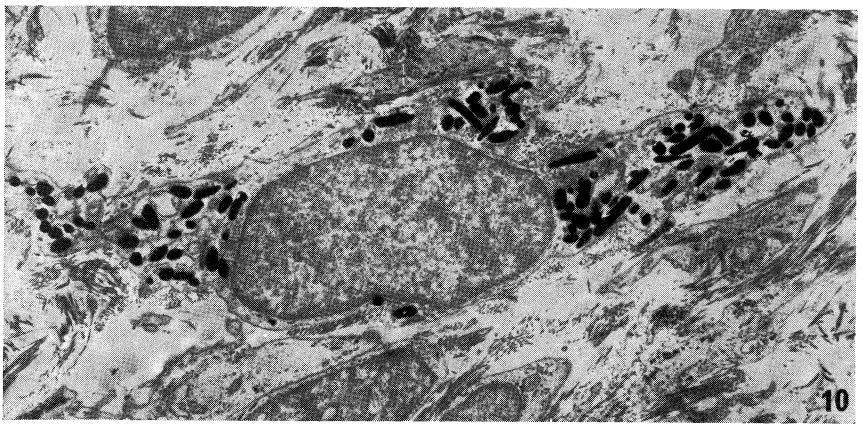


Fig. 8.—Vertical section of skin from a 54-day-old animal showing large dendritic melanocytes in the mid-level of the dermis. Silver impregnation. ×220.

Fig. 9.—Section (50 μm thick) of skin from a 97-day-old animal showing a large dermal melanocyte near a nerve (arrow) in the mid-level of the dermis. Acetylcholinesterase. ×700.

Fig. 10.—Electron micrograph of a dermal melanocyte in a 55-day-old animal. Uranyl acetate and lead citrate. ×4,600.

Melanocytes in the dermis, most common in samples taken 30–80 days after birth (Fig. 1), are usually sparse after 80 days and absent after 450 days. Except for the melanocytes seen in the upper part of the dermis, near the rudiments of the first hair follicles (see below), the dermal population is confined to the mid-level of the dermis (Fig. 8), usually near the main nerves (Fig. 9) in this region. These melanocytes are larger than those in the epidermis and do not discharge their melanin granules (Fig. 10), which are similar in shape but larger than those in epidermal melanocytes.

An unusual feature of several whole mount preparations of skin is the presence of paired dermal melanocytes (Fig. 11) when the dermal population is sparse. Very occasionally some of the dermal melanocytes appear to be arranged in groups of three or four.

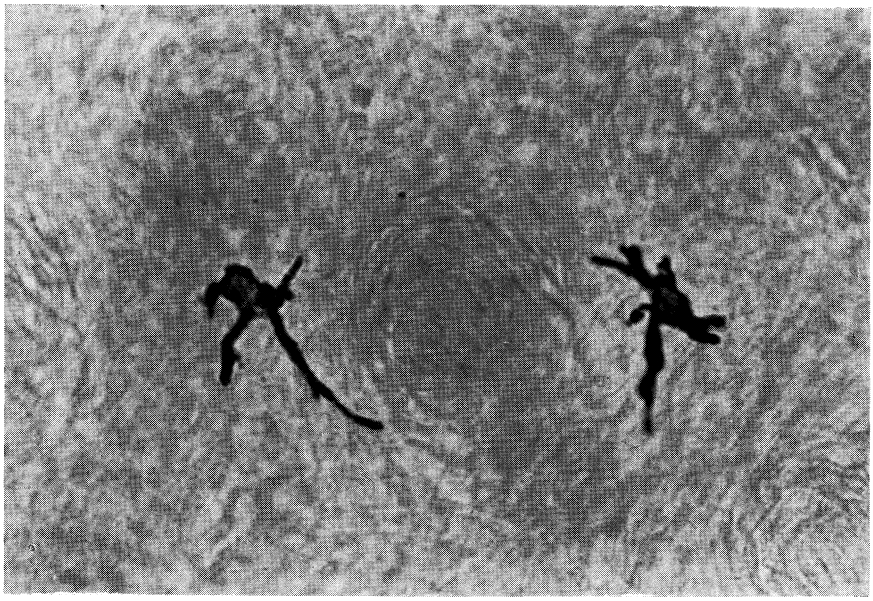
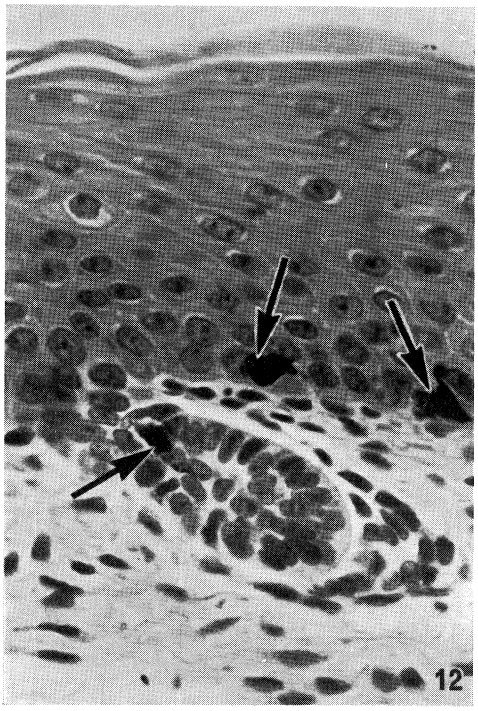


Fig. 11.—Whole mount preparation of skin from a 57-day-old animal showing an isolated pair of dermal melanocytes. Carmine. ×560.

(*b*) *Melanocytes and Hair Follicle Development*

Melanocytes in the epidermis are revealed only during the period of hair follicle initiation from the epidermis, that is, from 2 to 100 days after birth. Various types of follicles develop during this period. The first follicles begin their development mainly during the period from 2 to 20 days after birth. When fully developed these follicles are very small and grow minute pigmented hairs (total length about 0·5 mm) during the first hair cycle, which lasts only 2–3 weeks.

DOPA-reactive melanocytes are seen in the epidermis near the earliest follicle primordia detected 2 days after birth. At least some of the first-formed follicles appear to carry down with them some of the melanocytes already present in the epidermis (Fig. 12). In samples from several animals, from 7 to 11 days old (including one DOPA-incubated sample), melanocytes are not revealed in the epidermis (Fig. 1) although they are present in the bulbs of the most advanced follicles, which have reached the hair cone stage.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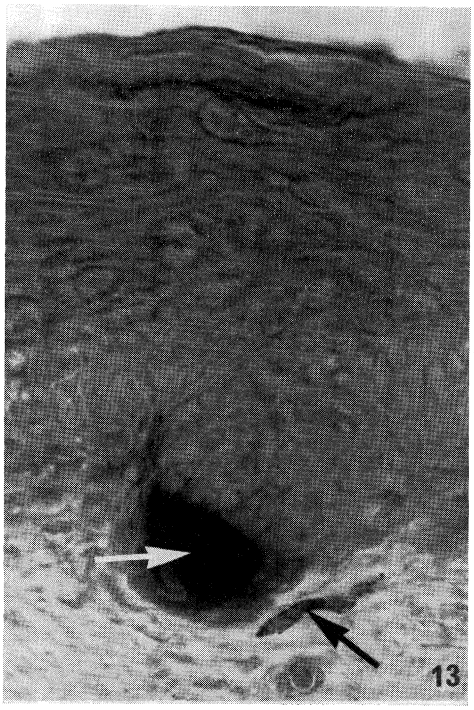
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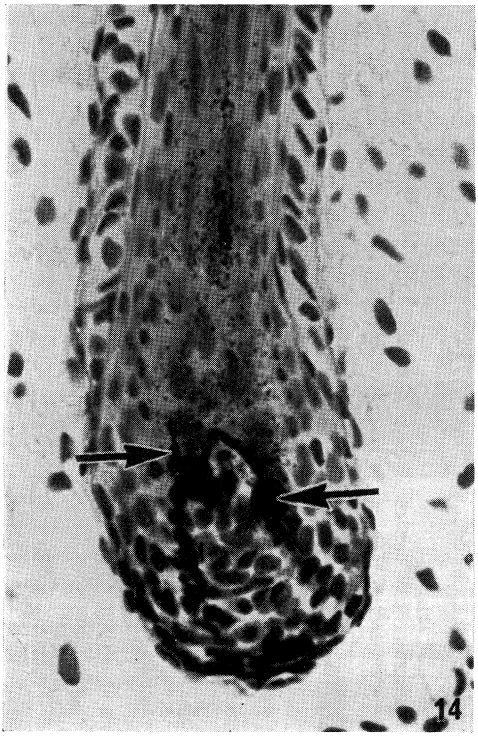
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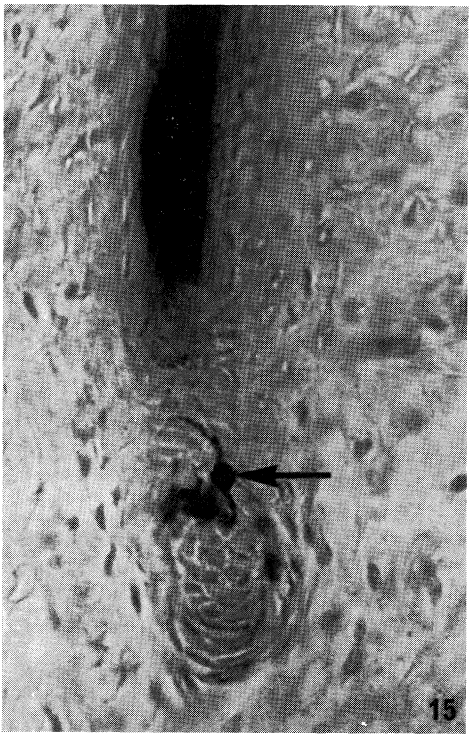
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Although dermal melanocytes are not uncommon near the primordia of the first-formed follicles (Fig. 13), no direct evidence has been obtained that these cells enter the follicle bulbs. The melanocytes responsible for hair pigmentation are confined to the epithelial region of the follicle adjacent to the upper part of the dermal papilla (Fig. 14). In addition, melanocytes are sometimes located at the base of the follicle bulb but are usually not seen until the follicle is growing a pigmented hair. These melanocytes, located either between the epithelial cells at the base of the bulb or in the dermis adjacent to the dermal papilla, are rounded or have short dendrites. At the end of the first hair cycle, some bulb melanocytes remain near the resting dermal papilla (Fig. 15). Examination of whole mount preparations of skin with quiescent follicles usually reveals three, four, or five melanocytes in this region.

The initiation of later populations of follicles from the epidermis begins at about 40 days after birth. Before and during this time melanocytes are common or dense in the epidermis (Fig. 1). All of these later-formed follicles appear to carry down epidermal melanocytes (Fig. 16) and subsequently form pigmented hairs. Occasional melanocytes are seen at the base of the follicle bulb (Fig. 17). At the end of each hair cycle these follicles also retain some melanocytes in the epithelial region near the resting dermal papilla (Fig. 18).

Subsequent to 100 days after birth there are no melanotic melanocytes in the epidermis (Fig. 1) or in the follicles, other than those in the region of the dermal papilla (Fig. 17). For a period of about 80 days, many additional follicles develop immediately below the level of the sebaceous gland ducts by branching from previously formed follicles. This branching results in the formation of large bundles of follicles. Melanocytes are not detected in the region of the dermal papilla of these branched follicles until the hair cone stage of development, after which all of these branched follicles grow pigmented hairs. Dermal melanocytes are occasionally seen near developing branched follicles but they are not seen entering the bulbs.

IV. Discussion

Melanotic melanocytes are present in the epidermis on the dorsal and lateral aspects of the body of this marsupial only during the period of follicle initiation from the epidermis. Thus, the present study supports the findings of Bolliger and Hardy (1945) that the skin of advanced pouch young is pigmented and that the epidermis of the adult lacks pigment. In some other mammals (e.g. bushbabies, Yun and Montagna 1965; black mice, Quevedo *et al.* 1966) the melanocytes are melanogenically active

Fig. 12.—Vertical section of the upper part of skin from a 25-day-old animal showing melanocyte (lower arrow) in an early-stage follicle of the first population. Two melanocytes (upper arrows) are seen in the basal layer of the epidermis. Haematoxylin and eosin. ×560.

Fig. 13.—Vertical section of the upper part of skin from a 14-day-old animal. A dermal melanocyte (right arrow) is near the alkaline phosphatase-positive dermal papilla cells (left arrow) below a follicle rudiment. ×560.

Fig. 14.—Longitudinal section of the lower part of a first-formed follicle growing a pigmented hair in a 14-day-old animal. The tip of the hair (not shown) is at the level of the epidermis. Melanocytes (arrows) cap the dermal papilla. DOPA reagent. ×560.

Fig. 15.—Longitudinal section (50 μm thick) of the lower part of a first-formed follicle which has completed its first hair cycle in a 21-day-old animal. Melanocytes (arrow) are in the region immediately above the resting dermal papilla. Silver impregnation. ×560.

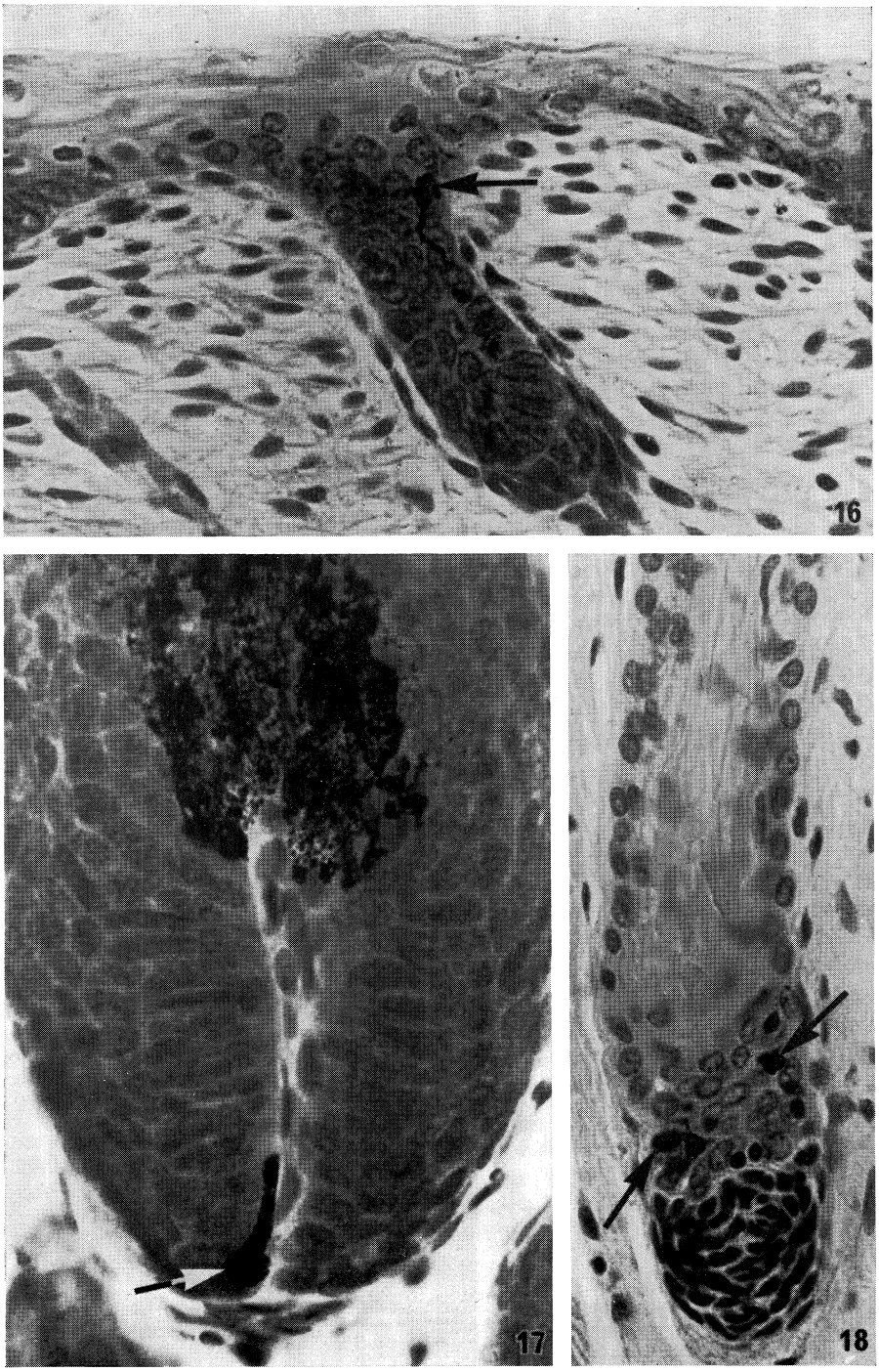

Fig. 16.—Vertical section of the upper part of the skin showing an immature follicle in a 71-day-old animal. Note the dendritic melanocyte (arrow) in the upper part of the follicle plug. Haematoxylin and eosin. ×560.

in young animals, but later persist in an amelanotic condition. The present observations indicate that most of the melanocytes which appear in the epidermis of *T. vulpecula* are eventually incorporated in the developing hair follicles. Very few melanocytes appear to move to the skin surface to be shed with the cornified cells of the epidermis.

Although melanocytes are present in the dermis for much longer than in the epidermis, they are never numerous. The pairs and small groups of dermal melanocytes seen in several samples suggest that some of these cells remain in close proximity after division. Although it is not possible to state definitely that dermal melanocytes enter follicle bulbs, the location of melanocytes in the dermis near the rudiments of the first-formed follicles suggests that this might occur. Dermal melanocytes are also occasionally seen near immature branched follicles. The absence of melanotic melanocytes in branched follicles before the hair cone stage of development suggests that these follicles carry down a complement of precursor melanocytes or that precursor melanocytes enter from the dermis.

An electron microscope study of the development of the epidermis in *T. vulpecula* (Lyne, Henrikson, and Hollis, unpublished data) has revealed occasional cells which may be precursor melanocytes between the keratinocytes of the basal layer. These cells lack the characteristic filaments of keratinocytes and they are not attached to the adjacent keratinocytes by desmosomes or to the basement membrane by hemidesmosomes. These features are characteristic of melanocytes and Langerhans cells (Brody 1969). To date, Langerhans granules, described in Langerhans cells in other species, have not been detected in *T. vulpecula*.

V. Acknowledgments

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VI. References

Bolliger, A., and Hardy, M. H. (1945).—The sternal integument of *Trichosurus vulpecula*. *J. Proc. R. Soc. N.S.W.* **78**, 122–33.

Brody, I. (1969).—The epidermis. In "Handbuch der Haut- und Geschlechtskrankheiten". (Eds. O. Gans and G. K. Steigleder.) Vol. 1. Pt. 1. pp. 1–142. (Springer-Verlag: Berlin.)

Gibbs, H. F. (1938).—A study of the development of the skin and hair of the Australian opossum, *Trichosurus vulpecula*. *Proc. zool. Soc. Lond.* **108**, 611–48.

Lyne, A. G. (1957).—The development and replacement of pelage hairs in the bandicoot *Perameles nasuta* Geoffroy (Marsupialia: Peramelidae). *Aust. J. biol. Sci.* **10**, 197–216.

Lyne, A. G., and Hollis, D. E. (1968).—The skin of the sheep: a comparison of body regions. *Aust. J. biol. Sci.* **21**, 499–527.

Fig. 17.—Longitudinal section of the lower part of a fully-developed follicle showing melanocytes capping the dermal papilla and a single melanocyte (arrow) at the base of the bulb. Sample from a 145-day-old animal. Haematoxylin. ×700.

Fig. 18.—Longitudinal section of the lower part of a resting follicle in a 435-day-old animal. Rounded melanocytes (arrows) are seen in the epithelial region near the resting dermal papilla. Haematoxylin, eosin, and picric acid. ×560.

LYNE, A. G., PILTON, P. E., and SHARMAN, G. B. (1959).—Oestrous cycle, gestation period and parturition in the marsupial *Trichosurus vulpecula*. *Nature, Lond.* **183**, 622–3.

LYNE, A. G., and VERHAGEN, A. M. W. (1957).—Growth of the marsupial *Trichosurus vulpecula* and a comparison with some higher mammals. *Growth* **21**, 167–95.

QUEVEDO, W. C., JR., YOULE, M. C., ROVEE, D. T., and BIENIEKI, T. C. (1966).—The developmental fate of melanocytes in murine skin. In "Symposium on Structure and Control of the Melanocyte". 6th Int. Pigment Cell Conf. pp. 228–41. (Springer-Verlag: Berlin.)

YUN, J. S., and MONTAGNA, W. (1965).—The skin of primates. XXV. Melanogenesis in the skin of the bushbabies. *Am. J. phys. Anthrop.* **23**, 143–8.