DEVELOPMENT OF THE EPIDERMIS OF THE MARSUPIAL *TRICHOSURUS VULPECULA*

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Summary

Development of the epidermis has been studied in the brush-tailed possum *T. vulpecula*. Although the epidermis is thin at birth (about 12 μm), there is a four- to fivefold increase in epidermal thickness during the first 21 days after birth. However, when the young first emerge from the pouch, at about 120 days, the epidermis has returned to the thickness seen at birth. The thickness in adults is usually about 15 μm. Hair follicles differentiate from the epidermis of pouch young throughout the period of increasing epidermal thickness and during most of the subsequent period of decreasing thickness.

Marked changes in the structure of the epidermis occur during the first few weeks after birth. Present at birth is a partially cornified periderm which is replaced by a fully cornified layer during the period of increasing epidermal thickness. When the maximum thickness is reached the epidermis has an extremely thick spinous layer. The adult epidermis is composed of a thin Malpighian layer and a desquamating cornified layer.

Some features of the structure and development of the epidermis of *T. vulpecula* are compared with those in other mammals. The process of keratinization is similar to that described in other species. In addition to melanotic melanocytes, cells which may be precursor melanocytes are seen in the epidermis up to 100 days after birth.

I. Introduction

In a study of the melanocytes in the skin of the brush-tailed possum, *Trichosurus vulpecula*, Lyne (1970) drew attention to the meagre information available on the integument of this marsupial. Only two earlier references to the epidermis in *T. vulpecula* are known to the authors. Gibbs (1938) referred to the structure and thickness of the epidermis in pouch young of unknown age and Henrikson (1969) published a note on the ultrastructure of the epidermis in the adult.

The present observations are mainly concerned with the postnatal changes in the epidermis in animals of known age and provide a necessary background for more detailed studies.

II. Material and Methods

The skin samples examined were from the 41 animals referred to previously (Lyne 1970). This material included samples from two embryos which, at 15 and 18 days after mating, were almost at term as the gestation period in *T. vulpecula* is 17–18 days (Lyne, Pilton, and Sharman 1959). All skin samples were taken from the dorsal and lateral aspects of the trunk.

Skin samples for electron microscopy were taken from five pouch young (2, 14, 21, 55, and 106 days after birth) and two adults (animals more than 1 yr old). These samples were fixed in osmium tetroxide buffered with veronal acetate or collidine (pH 7·2–7·4), or in glutaraldehyde buffered with cacodylate (pH 7·2–7·4), dehydrated in ethanol, and embedded in Araldite. Thin sections were stained with lead citrate and uranyl acetate. Other sections (1·0–1·5 μm thick) were stained with 0·1% aqueous azure A for light microscopy.

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Paraffin sections (8 μm thick) cut perpendicular to the skin surface and stained with haematoxylin, eosin, and picric acid were used for the measurements of epidermal thickness by the method of Lyne and Hollis (1968). Additional sections were subjected to the periodic acid–Schiff (PAS) reaction.

III. Observations

(a) *Changes in Epidermal Thickness*

Figure 1 shows the relation between epidermal thickness and age up to 500 days after birth, based on measurements of samples from the dorsal and lateral aspects of the trunk. The epidermis increases in thickness markedly during the early

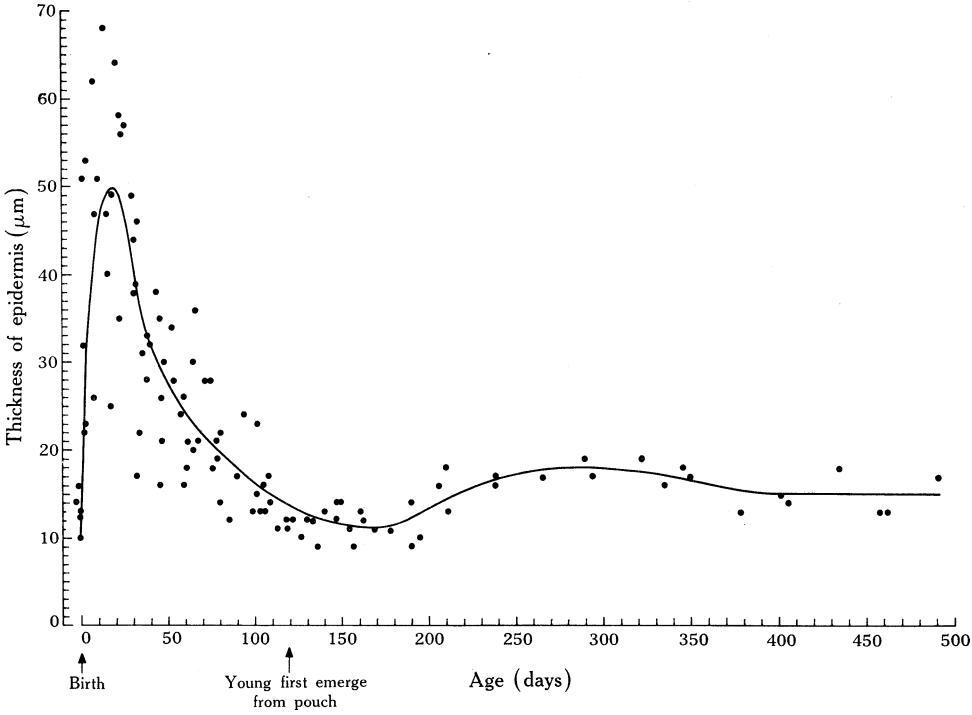

Fig. 1.—Relation between epidermal thickness and age, based on measurements of 112 skin samples from the dorsal and lateral aspects of the trunk of 29 animals. The curve has been drawn free-hand.

period of pouch life; it is about 12 μm thick at birth and shows a four- to fivefold increase in thickness by about 20 days of age. When the young first emerge from the pouch, at about 120 days after birth, the epidermis has decreased to the thickness seen at birth. There is a small increase in thickness at weaning, which occurs around 200 days. The epidermal thickness in adults is about 15 μm.

Fig. 4.—Section of skin from a new-born young. The epidermis (bracket) is about 12 μm thick and has a well-defined periderm. Arrows indicate large blood vessels containing nucleated cells in the dermis. Haematoxylin. $\times$560.

Fig. 5.—Section of epidermis (av. thickness 32 μm) from a 2-day-old animal. Azure A. $\times$1,400.

Fig. 6.—Section of epidermis (av. thickness 64 μm) from a 21-day-old animal. Azure A. $\times$700.

Fig. 7.—Section of epidermis (av. thickness 12 μm) from a 133-day-old animal. Haematoxylin and eosin. $\times$560.

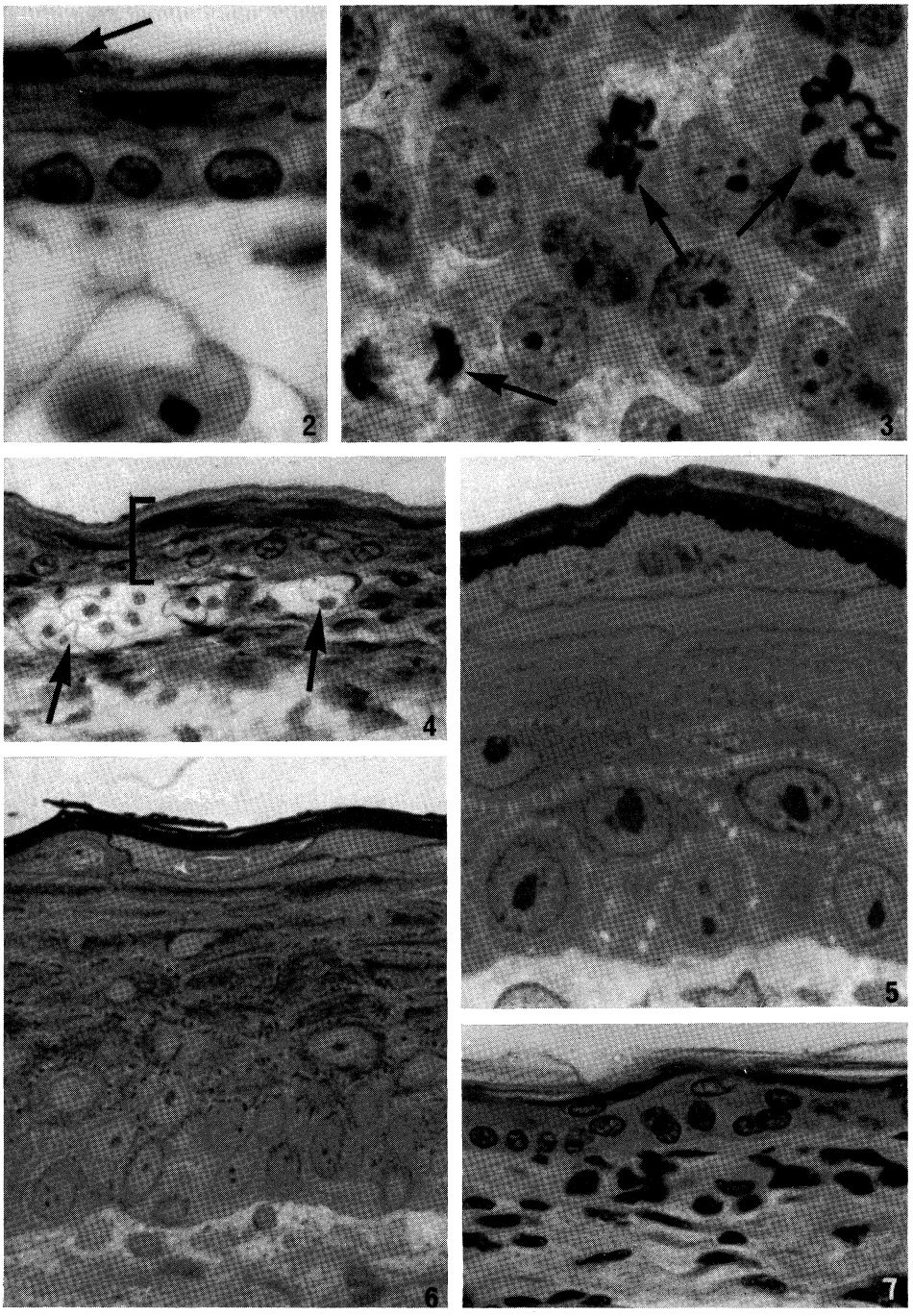

Fig. 2.—Vertical section of skin from a 15-day-old embryo. The epidermis is about 14 μm thick and has a relatively smooth junction with the dermis. Arrow indicates a nucleated cell of the periderm. Haematoxylin and eosin. $\times$1,400.

Fig. 3.—Whole mount preparation of skin from the embryo shown in Figure 2, viewed from above. The level of focus shows dividing cells (arrows) in the basal layer of the epidermis. Haematoxylin. $\times$1,400.

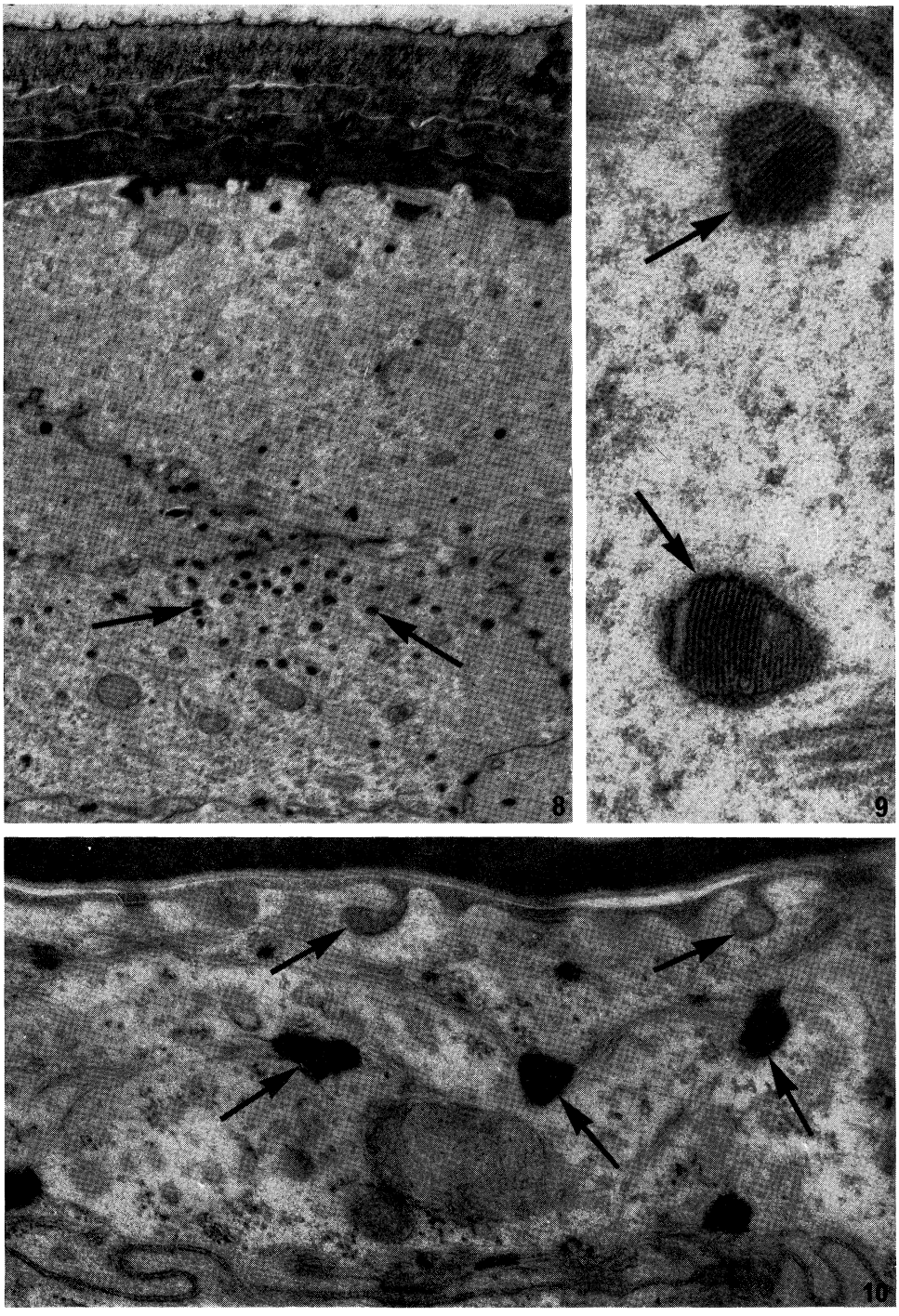

Fig. 8.—Vertical section of the upper part of epidermis from a 2-day-old animal showing membrane-coating granules (arrows) in a spinous cell. ×9,000.

Fig. 9.—Section of a spinous cell in a 21-day-old animal showing laminated membrane-coating granules (arrows). ×160,000.

(*b*) *Relation between Epidermal Thickness and Development of the Hair Follicles*

The period of rapid increase in epidermal thickness, which occurs soon after birth (Fig. 1), coincides with the period of initiation of the first population of hair follicles (Lyne, unpublished data). On the dorsal and lateral aspects of the body, the most advanced of these follicles begin their development at about 2 days after birth and grow rapidly, completing their first hair cycle in 2–3 weeks. Later populations of follicles are initiated from the epidermis during the time of decrease in thickness of the epidermis. Most of these follicles develop between 40 and 100 days after birth. During the period from 110 to 180 days after birth, when many additional follicles develop by branching from previously formed follicles, the thickness of the epidermis is consistently thin.

(*c*) *Structure of the Epidermis*

In the 15-day-old embryo the differentiation of the epidermis is well advanced and there is a relatively smooth junction between the epidermis and the dermis (Fig. 2). The epidermis is composed of a basal layer with cuboidal cells, an intermediate layer of flattened cells (about two cells thick), and a periderm or epitrichium, also of flattened cells with distinct nuclei and a granular cytoplasm. Cell divisions in the basal layer of this 15-day-old embryo (Fig. 3) and in the new-born young are numerous. The epidermis of the new-born young (Fig. 4) is slightly thinner than the dermis which has a network of blood vessels containing nucleated cells.

The rapid proliferation of basal cells and resulting increase in thickness of the epidermis is apparent at 2 days after birth (Fig. 5). The epidermis, after reaching its maximum thickness at about 21 days after birth, is characterized by an extremely thick spinous layer (Fig. 6). Samples taken from older animals show fewer cell divisions in the basal layer. In advanced pouch young and adults the spinous layer is only one to two cells thick (Fig. 7). No glycogen has been revealed in the epidermis of the pouch young or adults.

(i) "*Membrane-coating Granules*".—Granules (Fig. 8), which resemble the membrane-coating granules described by Matoltsy and Parakkal (1967), are found in all the material examined with the electron microscope. These granules are located in the spinous and granular layers, particularly in the former. Figure 9 shows the internal lamellae which are characteristic of membrane-coating granules. In the thick epidermis of the 21-day-old animal, these granules are identified in four to five layers of spinous cells, whereas in the thinner epidermis of advanced pouch young and adults, the granules in the spinous layer are usually restricted to the cells adjacent to the granular layer. Lamellar structures which resemble membrane-coating granules are often seen attached to the distal plasma membrane of granular cells adjacent to the cornified layer (Fig. 10). The lamellae of these structures are best seen in adult epidermis.

(ii) *Keratohyalin Granules*.—Keratohyalin granules are present in the cytoplasm of granular cells of all animals examined with the electron microscope. They are

Fig. 10.—Vertical section of the upper part of the epidermis of an adult animal showing the junction between the granular and cornified layers. The upper arrows show lamellar structures (lamellae not clearly seen at this magnification) attached to the distal surface of the plasma membrane of a granular cell. The lower arrows show keratohyalin granules and their associated filaments. ×60,000.

small ($< 1\ \mu m$ in diameter) and sparsely distributed in the younger animals (2, 14, and 21 days old), and larger ($< 5\ \mu m$ in diameter) and more numerous in the older animals. At all ages there is an obvious association of these granules with filaments (Fig. 10).

(iii) *Cornified Layer.*—In the 2-day-old animal the cells of the periderm are incompletely cornified (Fig. 11). These cells, which are nucleated, show a thickened outer border without desmosomes. The plasma membrane of the free surface is a double membrane (Fig. 11, inset). No keratohyalin granules are present in these cells. Beneath the periderm there are several layers of keratinizing cells (Fig. 11).

In the samples from older animals, a stratum corneum of fully cornified cells is present. The opacity of these cells varies but generally the superficial cells are less electron-dense. The plasma membrane of cornified cells is thickened in all samples examined. There is a "keratin pattern" in the proximal cornified cells of the 106-day-old animal and in the adults (Fig. 12). In this figure the relatively wide intercellular space between the keratinized cells may be an artifact.

(*d*) *Melanocytes and Precursor Melanocytes in the Epidermis*

Melanotic melanocytes in the epidermis are present only during the period from 2 to 100 days after birth (Lyne 1970). Samples from pouch young 2–55 days after birth contain an occasional cell without melanin granules (Fig. 13) which may be a precursor melanocyte between the keratinocytes of the basal layer. This cell lacks the characteristic filaments of keratinocytes and is not attached to the adjacent keratinocytes by desmosomes or to the basement membrane by hemi-desmosomes. These features are characteristic of melanocytes as well as of Langerhans cells (Brody 1969), but Langerhans granules are absent in these cells in *T. vulpecula.* No melanocytes or possible precursor melanocytes have been observed in the epidermis of the 106-day-old pouch young or the adults.

IV. Discussion

Although the epidermis of *T. vulpecula* is very thin at birth, there is a well-defined periderm or epitrichium similar to that described in the marsupial native cat *Dasyurus viverrinus* (Hill and Hill 1955). The periderm protects the new-born young from desiccation while crawling from the urogenital opening to the pouch. The plasma membrane of the free surface of the periderm cells is a double membrane similar to that described by Breathnach and Wyllie (1965) for the superficial cells of the epidermis of human foetuses at 12 and 14 weeks. Further studies are needed to determine the way in which this double membrane is formed.

The ages of the animals examined by Gibbs (1938), although not recorded by her, ranged from about 12 days to 6 months as estimated from the observations of Lyne and Verhagen (1957) and Lyne (unpublished data). The present observations on the thickness of the epidermis in *T. vulpecula* are in close agreement with those of Gibbs (1938) on the basis of the estimated ages. Although Gibbs did not examine very young animals, she noted the early increase and subsequent decrease in the thickness of the epidermis. Gibbs (1938) has shown that the epidermis of the

Fig. 12.—Section of the stratum corneum in an adult animal revealing "keratin pattern" with longitudinal and cross-sectional outlines of the filaments. The relatively wide intercellular space (arrow) may be an artifact. ×144,000.

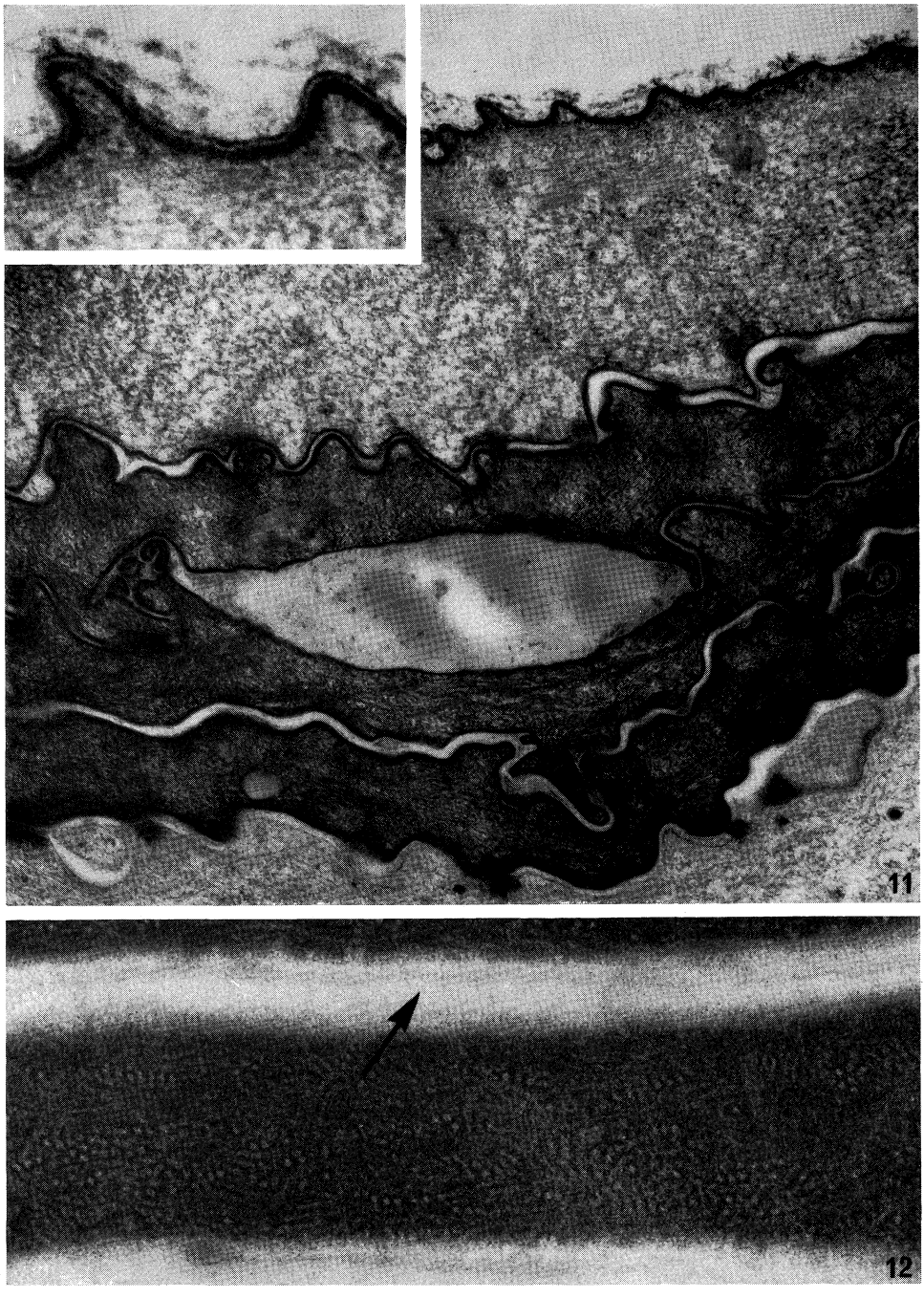

Fig. 11.—Vertical section of the outermost layers of the epidermis of a 2-day-old animal. An incompletely keratinized cell of the periderm is situated above several layers of keratinized cells. ×44,000. *Inset:* Thickened outer border of periderm cell. The plasma membrane of the free surface is a double membrane. ×143,000.

anterior dorsal region is thicker than that of the posterior dorsal region. It is reasonable to expect that the epidermis from dorsal and lateral body positions also differs and this is probably responsible for some of the variations in the thickness of the epidermis seen in animals of the same age (Fig. 1).

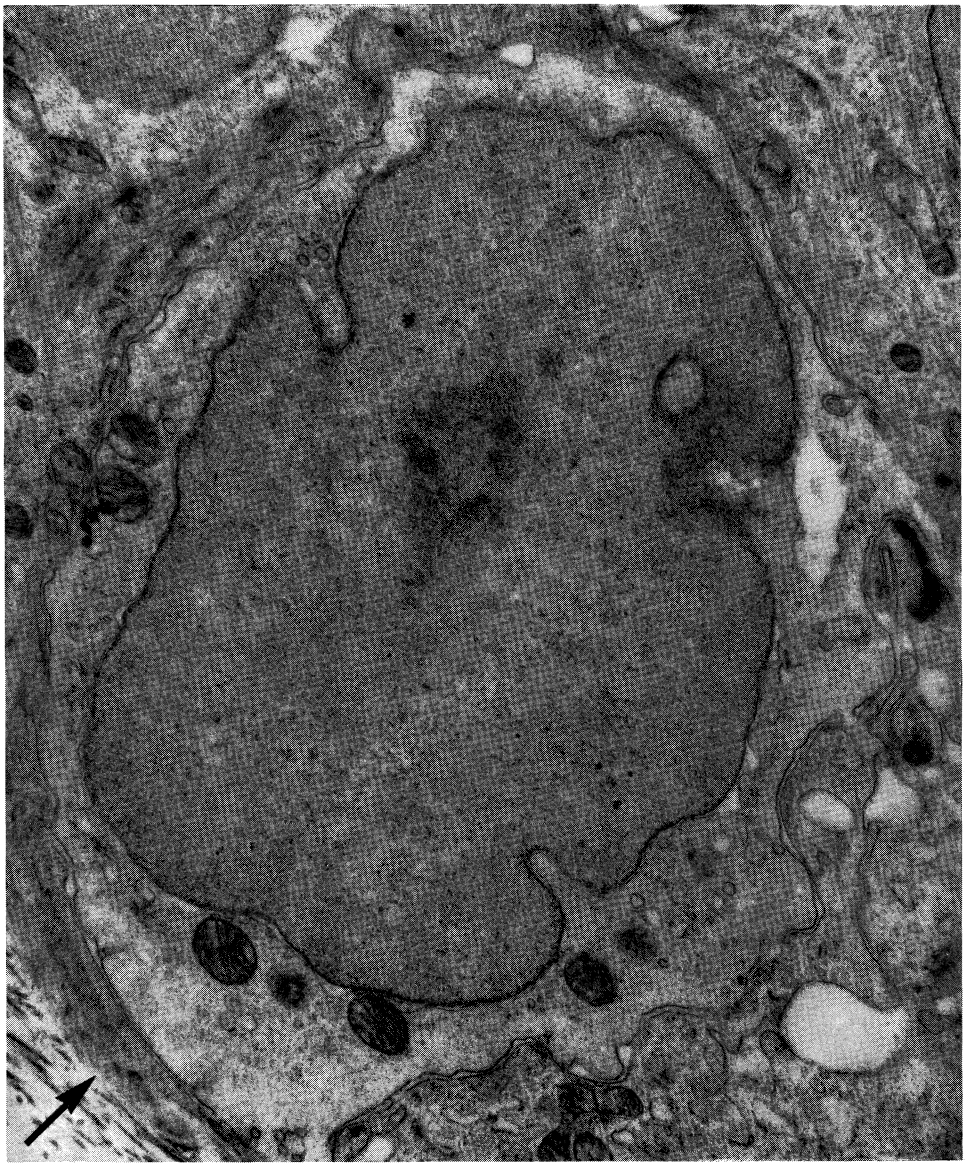

Fig. 13.—Section of cell which may be a precursor melanocyte in the basal layer of the epidermis of a 21-day-old animal. Arrow indicates basement membrane. ×20,000.

Follicle initiation from the epidermis occurs during the period of decreasing as well as increasing epidermal thickness. This phenomenon is also seen to some extent in the mouse (Slee 1962). In the ox (Lyne and Heideman 1959) the epidermis

increases and then decreases in thickness during foetal life, with follicle initiation coinciding with the period of increasing thickness.

The general features in the development of the epidermis of *T. vulpecula* are similar to those described for man (Montagna 1962), the rat and mouse (Hanson 1947), and other mammals. The process of epidermal cornification, already briefly described in *T. vulpecula* (Henrikson 1969), does not appear to differ from that observed in other species (Matoltsy and Parakkal 1967). The membrane-coating granules present in the epidermis of *T. vulpecula* are similar in distribution and structure to those described by Matoltsy (1966) in young red kangaroos.

T. vulpecula differs from various other mammals, for example the mouse (Hardy 1952), rhesus monkey (Bell 1969), and man (Pinkus 1958), in that glycogen is absent from the epidermis during all stages of hair follicle development. It is unknown whether or not glycogen is present in the epidermis during the early stages of its differentiation, which occur before birth. In the mouse, glycogen is present in large quantities in the embryonic epidermis when this consists of only one to three layers of cells (Hardy 1952).

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