

EMBRYOLOGY OF THE EPIDERMIS: ULTRASTRUCTURAL ASPECTS

III. Maturation and Primary Appearance of Dendritic Cells in the Mouse with Mammalian Comparisons

Larry W. Weiss and Alvin S. Zelickson

From the Department of Dermatology, University of Minnesota, Minneapolis, Minnesota, USA

Abstract. The epidermis of mice ranging in age from prenatal day 17 through postnatal day 4 and, in addition, postnatal day 18, was studied with the electron microscope. In the 17 day fetus, the periderm may or may not be present and a stratum corneum is developing in the latter case. The cells of the strata spinosum and granulosum contain much glycogen and many keratinosomes and homogeneous keratohyalin granules which appear to line up in a row near the distal cell membranes, fuse and form keratinized cells which contain material similar in density to that of the individual keratohyalin granules.

In the 18 to 20 day fetuses, no periderm is present. The stratum corneum becomes well-developed. The quantity of glycogen decreases but the number of keratinosomes and keratohyalin granules increases. Some cells in the basal region contain necrotic phagocytosed cells. Few changes occur in the epidermis of the neonate, although the stratum corneum increases in thickness. In the early postnatal period, the mouse epidermis is mature in appearance and resembles that of man. By postnatal day 18, the whole epidermis is much thinner, although all classical strata can usually be identified.

The primary appearance and subsequent maturation of epidermal dendritic cells was also studied in the mouse during the embryonic, fetal, early postnatal and 18 day postnatal periods. Melanocytes which lacked cell processes were first identified in the 15 day fetus. Melanocyte cell processes and an increasing number of melanosomes, as well as melanocytes, are present between prenatal day 18 and postnatal day 3. On postnatal day 4, fewer melanocytes are present and they contain vacuoles. Phagocytosed melanocytes are also present within basal keratinocytes.

Undifferentiated dendritic cells or indeterminate cells are observed beginning on prenatal day 16. These cells do not appear to increase significantly in number subsequently. On postnatal day 18, Langerhans cells and indeterminate cells are present but no melanocytes can be identified. No Merkel cells were observed. Mitotic dendritic cells or dendritic cells traversing the basal lamina were not observed.

Key words: Embryology; Epidermis; Keratinocyte; Dendritic cells; Melanocyte; Langerhans cell; Indeterminate cell

This third paper in the series on the detailed morphology of the developing mammalian epidermis covers the period from the time the stratum corneum is first formed through the early postnatal period during which time the epidermis achieves full maturation. During the first few days of postnatal life, the mouse epidermis is most similar to that of the human adult. A short time later, the mouse interfollicular epidermis becomes much thinner and remains so. This period of development of the mouse epidermis has been studied with both light (21, 59) and electron microscopes (13, 59). There have also been investigations of certain morphological aspects of the epidermis during parts of the late prenatal and perinatal periods (11, 15, 18, 33, 46, 47). The ultrastructure of the adult mouse interfollicular epidermis has been described in detail (52).

Comparable morphological features of the developing epidermis of the prenatal and postnatal rat have been described (1, 3, 5, 16, 21, 23, 29, 30). There are many similarities between developing mouse and human fetal epidermis and studies of certain periods during the latter part of human fetal epidermal development have been investigated and have contributed to a general understanding of the formation of a mature epidermis at the ultrastructural level (6, 7, 22, 25, 35, 51).

In conjunction with the study of the developing epidermis, the primary appearance and subsequent maturation of dendritic cells, namely the melanocyte, Langerhans cells and indeterminate or undifferentiated dendritic cell were studied in detail. The embryological origin of melanocytes from the neural crest (38-40) as well as their appearance and presence in the epidermis of perinatal mice and rats (36, 41, 42, 48, 57, 59, 60) has been the subject of numer-



Fig. 1. Survey of the 17 day fetal mouse epidermis. A stratum corneum (SC) is forming. B=basal cells, G=glycogen. $\times 5250$.

ous investigations. Similar studies of epidermal melanocytes have been made in humans (2, 6, 8, 10, 17, 22, 32, 34, 49, 66, 67). The indeterminate and Langerhans cells have also been extensively studied in mice (9, 36, 44, 58-60) and in humans (6, 8, 10).

MATERIALS AND METHODS

Skin biopsies of C57Bl/6 mouse fetuses 17-20 days of age, neonates, and postnatal mice 1-4 days of age were taken at 24 hour intervals and processed for electron microscopic studies. In addition, skin biopsies of postnatal mice 18 days of age were also taken for this study. The reader is referred to the first paper of this series for details of this procedure which is similar in all respects to that used in the present investigation (61). The studies of dendritic cells were done in conjunction with the complete series of investigations of epidermal development and therefore are of mouse skin taken at 24 hour intervals from animals ranging in age from prenatal day 8 through postnatal day 4 and in addition, postnatal day 18.

OBSERVATIONS

Keratinocytes

17 day fetus

The basal aspect of the epidermis is irregular and the basal lamina is well developed. Numerous mature hemidesmosomes are present. There are three to four layers of cells in the stratum spinosum and four to five layers in the stratum granulosum. A layer of periderm may or may not be present (Fig. 1). When present, the peridermal cells are very flattened except in the region of the nuclei. There are few desmosomes between the periderm and stratum granulosum but desmosomes are often present between individual peridermal cells. The stratum corneum, if present, contains one to three layers of flattened cells.

Numerous mitotic figures are present in the basal layer. Both hemidesmosomes and desmosomes remain intact during the mitotic process. Concentrations of glycogen are no longer present in the basal cells but the stratum spinosum contains glycogen aggregates. Circumferential bundles of tonofibrils are present between the perinuclear fibril-free zone and periphery of the spinous cells. Keratinosomes are particularly numerous in the distal cytoplasm of the upper spinous cells.

The cells forming the stratum granulosum become increasingly flattened as they move toward the surface. Concentrations of particulate glycogen, tonofibrils and keratinosomes are present. The cell membranes appear to be thickened, perhaps due to the close apposition of amorphous, dense material. Amorphous material is present also in the extracellular space of the upper portion of this layer. Keratinosomes, fused with the distal cell membrane, are often observed. Numerous homogeneous keratohyalin granules are present. Heterogeneous keratohyalin granules, which contain granular and lipid

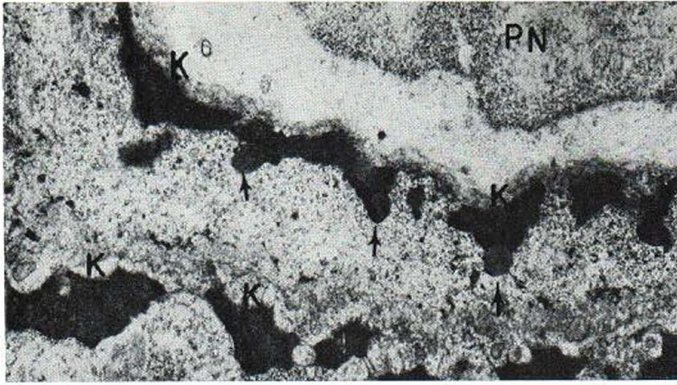


Fig. 2. Junction of the stratum granulosum and periderm of the 17 day fetus. Note that the keratohyalin (*K*) material appears to be dispersing and then coalescing again to form a continuous band along the superior cell membrane. Oval bodies (*arrows*), similar to those present in keratohyalin granules on day 16, are present in association with the more dense material. *PN*=peridermal cell nucleus. $\times 12\ 100$.

material, are observed only in the uppermost portion of the stratum granulosum. Intra-mitochondrial dense bodies, larger than those observed on day 16, are also present.

In the cell layer just below the uppermost layer of the stratum granulosum, keratohyalin granules, in which particulate material may be discerned, appear to be lining up in a row near the outermost portion of the cell (Fig. 2). These keratohyalin granules appear to be connected by fibrillar material and in the uppermost layer of the stratum granulosum, the keratohyalin granules are slightly larger and appear to be coalescing to form a continuous band (Fig. 3). Oval, lipid masses, often observed in the keratohyalin granules on day 16, are distinctly visible in the lower portion of this band of keratohyalin material. Another cell layer, peculiar to this stage of development, is located between the granular and cornified layers or periderm (Figs. 4-5). This may be considered to be a fetal transitional layer. The morphology of these cells resembles the cells of the stratum corneum in configuration and no organelles of any type are visible; they are filled with very dense material. The intracytoplasmic material adjacent to the upper and lower cell membranes is particularly electron-dense. The central portion of these

cells contains less-dense amorphous material in which material of a greater density is occasionally present.

If the periderm is absent, a thin stratum corneum is present (Fig. 5). These cornified cells are long and narrow and have an internal morphology which differs somewhat from that of mature cornified cells. The peridermal cells contain little more than nuclei, finely particulate material and vesicles. This is the last day in which any periderm is observed.

18 to 20 day fetuses

The epidermis of the 18 day fetus is much more mature (Fig. 6). Glycogen is present in the stratum spinosum and lower stratum granulosum but the quantity has decreased from day 17. Occasionally, some extracellular glycogen is present. Both keratinosomes and keratohyalin granules are more numerous and larger bundles of tonofibrils are present. The distinct morphological changes which are seen on day 17 in the upper stratum granulosum are no longer evident and there is an abrupt change from the granular to the horny layer. Much intercellular material and material adjacent to the cytoplasmic side of the dense cell membranes is present in this

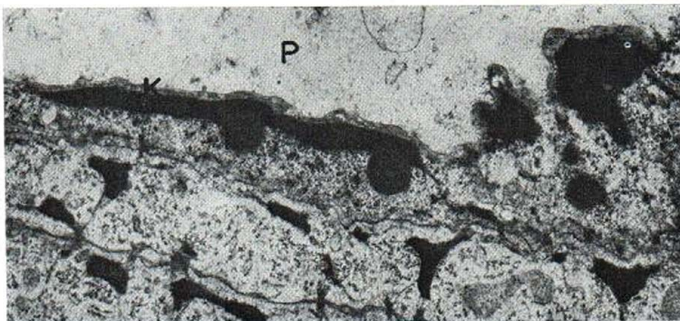


Fig. 3. Junction of the stratum granulosum and periderm in the 17 day fetus. Note that the keratohyalin (*K*) material is forming a more continuous band of material than that shown in Fig. 2. *P*=periderm. $\times 12\ 100$.

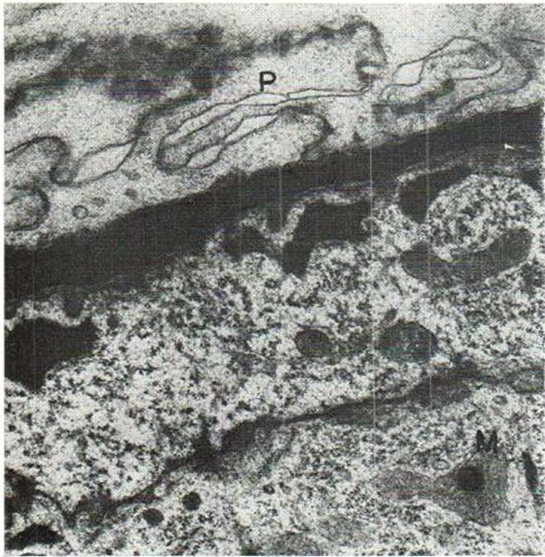


Fig. 4. Junction of the stratum granulosum and periderm (P) in the 17 day fetal mouse epidermis. Note the continuous band of dense material filling the cell just below the periderm. Desmosomes are present between the portions of the two peridermal cells shown. An intra-mitochondrial (M) dense body is also present in the lowermost granular cell. $\times 17\ 800$.

region. Composite desmosomes are often present between the stratum granulosum and stratum corneum and modified desmosomes are present between the cornified cells.

The stratum corneum is now five to seven layers in thickness and the cells contain dense areas of keratohyalin material. The epidermis of the 19 day

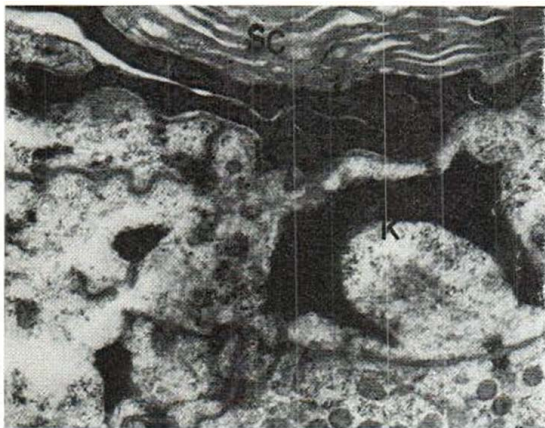


Fig. 5. Junction of the strata corneum (SC) and granulosum of the 17 day fetus. A multi-layered, immature stratum corneum has formed above the dense, keratinized cells and no periderm is present. K=keratohyalin material. $\times 23\ 000$.

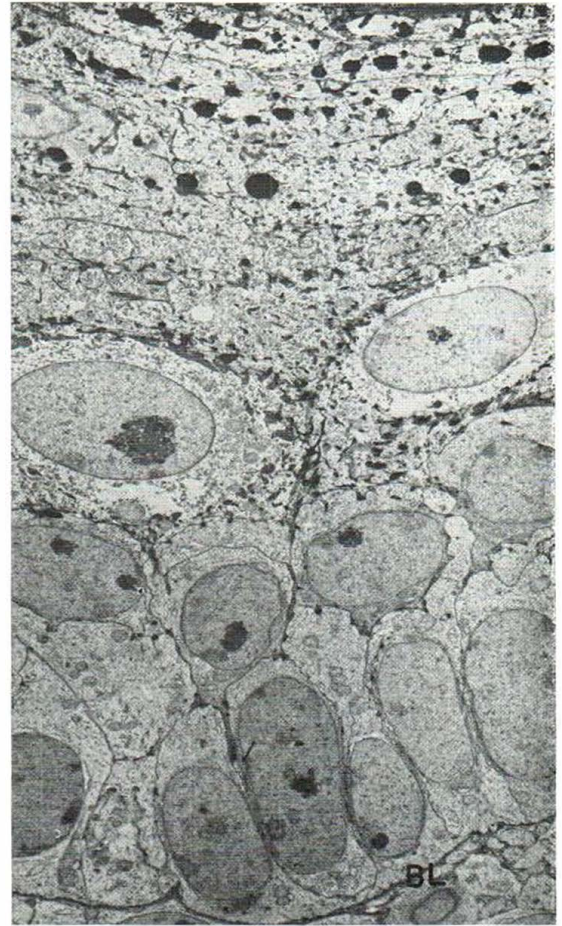


Fig. 6. Survey of the 18 day fetal mouse epidermis showing all layers below the stratum corneum. BL=basal lamina. $\times 3\ 700$.

fetus differs little from day 18. There are ten or more cell layers in the stratum corneum. Dense keratohyalin material is still present in the lowermost cornified cells (Fig. 7). On day 20, fifteen or more cell layers form the stratum corneum but the stratum spinosum often has only two to three layers. Little intra- or extra-cellular glycogen remains, although a small amount may be present in the stratum spinosum.

One particular observation of the 20 day fetal epidermis deserves further comment. Cells in the basal and lower spinous layer sometimes contain large, membrane-limited inclusions (Fig. 8). These inclusions appear to be degenerative cells which have been phagocytosed. Often nuclear remnants, organelles and other particulate material is identifiable. Cells in the upper stratum spinosum occasionally

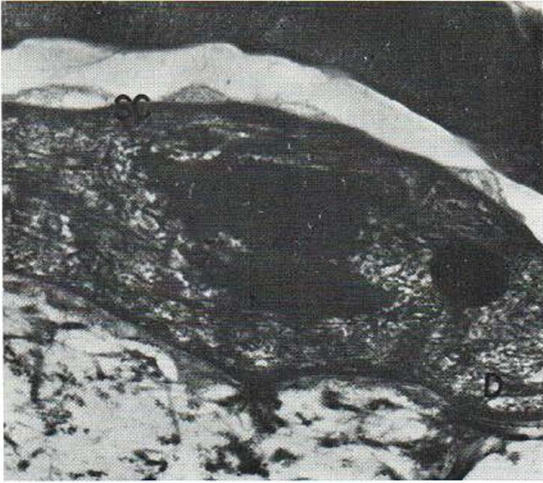


Fig. 7. Junction of the strata granulosum and corneum (SC) in the 19 day mouse fetus. Homogeneous keratohyalin material persists in the lowermost cornified cells. *D*=desmosome. $\times 24\ 500$.

contain smaller vacuoles filled with granular material which may be cellular remains of incompletely digested cells.

0 day or neonatal animal

Most mice were born on day 21 ± 12 hours and were designated as day zero. No morphological changes were noted in the epidermis in relation to the passage from a fluid to a gaseous environment. The epidermis is composed of a basal layer, one to two layers of prickly cells, three to four layers of granular cells and about twenty layers of cornified cells. Occasional mitotic figures are present in the basal and lower spiny layers. More keratinosomes and tonofibrils appear to be present in the stratum granulosum than on the previous two days. Occasional nucleated cells, surrounded by a perinuclear ring of keratohyalin granules, are present in the upper portion of the stratum granulosum. The cell membranes appear to be thickened in this region and some amorphous extracellular material is present. The flattened horny cells exhibit a characteristic keratin pattern. Occasional small areas of dense keratohyalin material are present in the cytoplasm of the lowermost cornified cells.

1 to 4 day postnatal animals

The general morphology of the epidermis is similar to that of the neonate although the strata granulo-

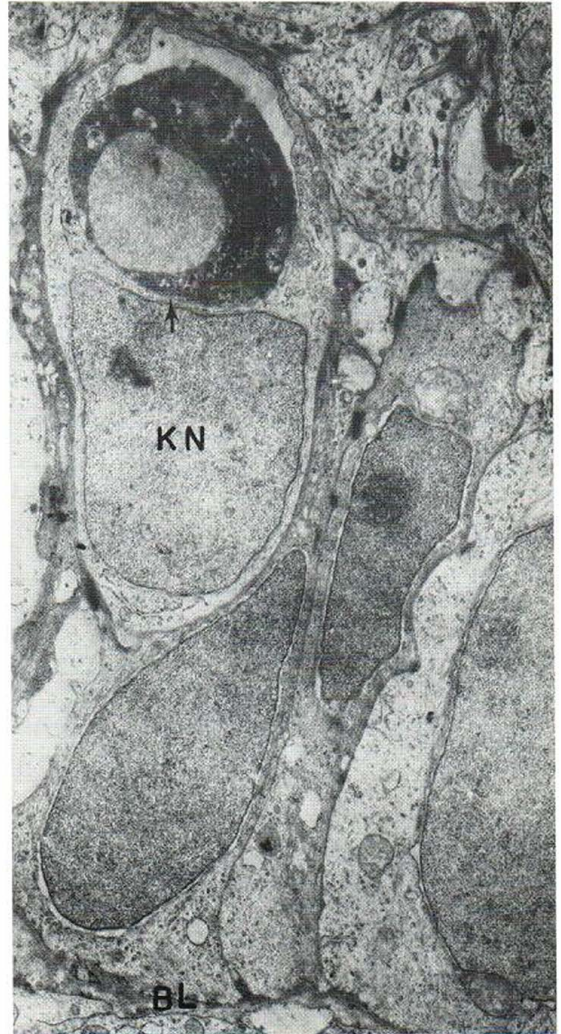


Fig. 8. Survey of the lower portion of the 20 day fetal mouse epidermis. Note the phagocytic inclusion (arrow) in the basal cell. *BL*=basal lamina. *KN*=keratinocyte nucleus. $\times 12\ 900$.

sum and corneum appear to be slightly thicker. Melanin is present in some basal cells on day one and thereafter. In the region of the stratum granulosum, there appears to be an increase in the amount of extracellular amorphous material. Keratinosomes are often observed in communication with the extracellular space. Differences in the keratin pattern, related to the proximal, intermediate and distal regions of the stratum corneum are noted.

18 day postnatal animal

The whole interfollicular epidermis has become much thinner than it was during the early postnatal



Fig. 9. Melanocytes are first observed in the 15 day mouse fetal epidermis. A portion is shown of one of these melanocytes which contains few immature melanosomes (arrows) and lacks cell processes. MN = melanocyte nucleus, BL = basal lamina. $\times 26\ 600$.

period. Occasionally, a basal cell contains a phagocytosed cell within its cytoplasm. There are fewer organelles in the cells of the stratum granulosum and both the number and size of the keratohyalin granules have decreased markedly. In the region between the upper stratum granulosum and lower stratum corneum, the cell membranes appear to be thickened and amorphous extracellular material is present. Here, this material appears to distend the extracellular space, especially between desmosomes. The stratum corneum is very irregular and the sublayers with their characteristic keratin patterns are less distinct.

Dendritic Cells

No dendritic cells are observed between prenatal days 8 through 14. Interfollicular epidermal melanocytes are observed for the first time in the 15 day fetus (Fig. 9). These melanocytes are sparse, have oval cell bodies, lack dendritic processes and con-

tain few immature melanosomes. On prenatal days 16 and 17, the number and location of the melanocytes in the basal and suprabasal regions is similar to that of day 15. These cells contain both unmelanized and partially melanized melanosomes. Indeterminate cells, identified by their lack of melanosomes, Langerhans granules, desmosomes and hemidesmosomes are observed for the first time in the 16 day fetus.

In the epidermis of the 18 and 19 day fetuses, the number of melanocytes appears to have increased and dendritic cell processes are present. The number of early stage melanosomes within the melanocytes has increased. A few indeterminate cells are present. In the 20 day fetus and in the neonate, more melanocytes appear to be present and they contain some stage IV melanosomes. During postnatal days 1 to 3 inclusive, the number of melanocytes and the number of melanosomes within them seems to have increased (Fig. 10). A corresponding increase in indeterminate cells does not occur.

On postnatal day 4, fewer melanocytes are present, Membrane-limited vacuoles are present near the Golgi apparatus and in other areas in these melanocytes (Fig. 11). Fewer melanosomes are present. Phagocytosed melanocytes, in which melanosomes and nuclear remnants can be identified, are occasionally observed completely within the cytoplasm of basal keratinocytes (Fig. 12). Epidermal melanocytes can not be identified on postnatal day 18 but a few indeterminate cells are present. Langerhans cells are observed only on postnatal day 18. At this age, the Langerhans cells are not numerous and are not observed above the stratum spinosum.

Mitotic dendritic cells were not observed, although, rarely, two dendritic cells were observed in close apposition. Such cells may have been early post-mitotic cells. Langerhans granules and melanosomes were not seen within the same dendritic cell. Dendritic cells were not observed traversing the basal lamina.

DISCUSSION

In the present study, the periderm was no longer observed after fetal day 17. These cells appear to lose their attachments to the underlying developing stratum corneum and desquamate into the amniotic fluid. Hanson (21) observed that the stratum corneum develops on day 16 and that the periderm is not present after day 17 in the mouse. These ob-

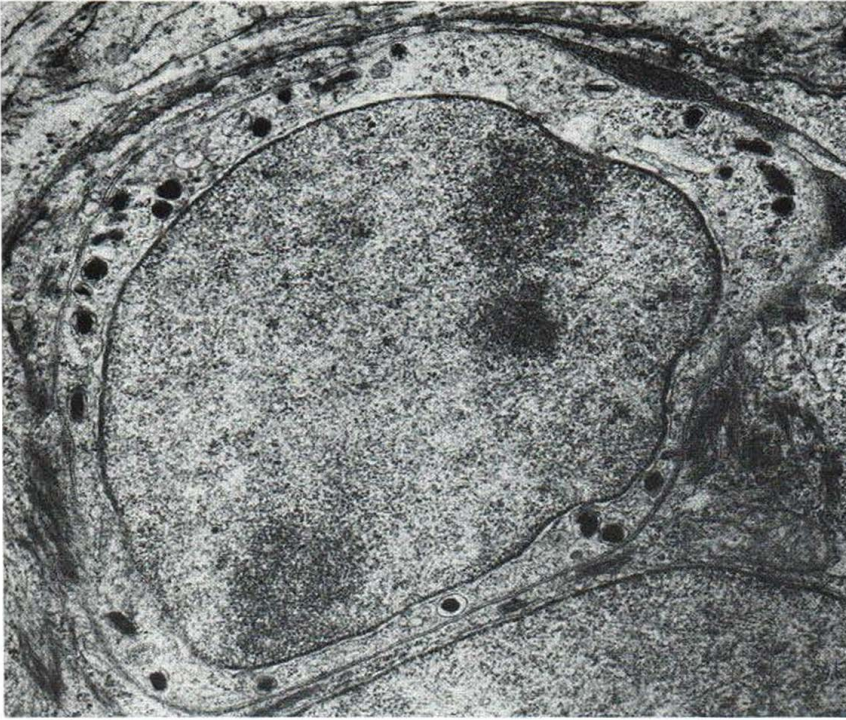


Fig. 10. Numerous epidermal melanocytes are present on postnatal days one to three. These melanocytes have many processes and contain melanosomes in all stages of development. Day 2 postnatal mouse epidermis. $\times 19\ 500$.

servations are in general agreement with other ultrastructural studies of the developing stratum corneum from the sixteenth to the seventeenth day (11, 13). In the rat, periderm persists throughout

fetal life (3, 16) although a well-defined stratum corneum forms beneath it during this period. In rat epidermis, fixed with glutaraldehyde followed by osmium, heterogeneous keratohyalin granules can

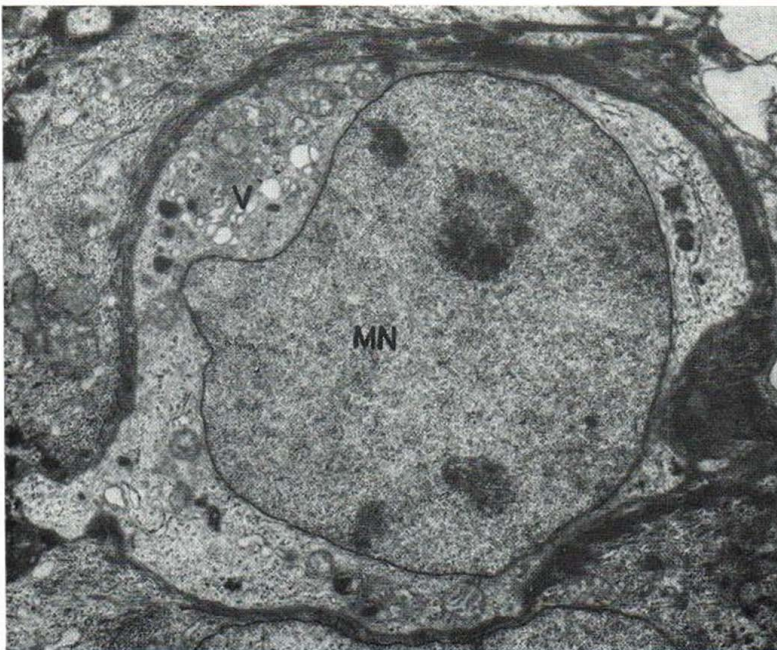


Fig. 11. On postnatal day 4, melanocytes contain few melanosomes but have numerous vesicles (V). MN = melanocyte nucleus. $\times 8\ 050$.

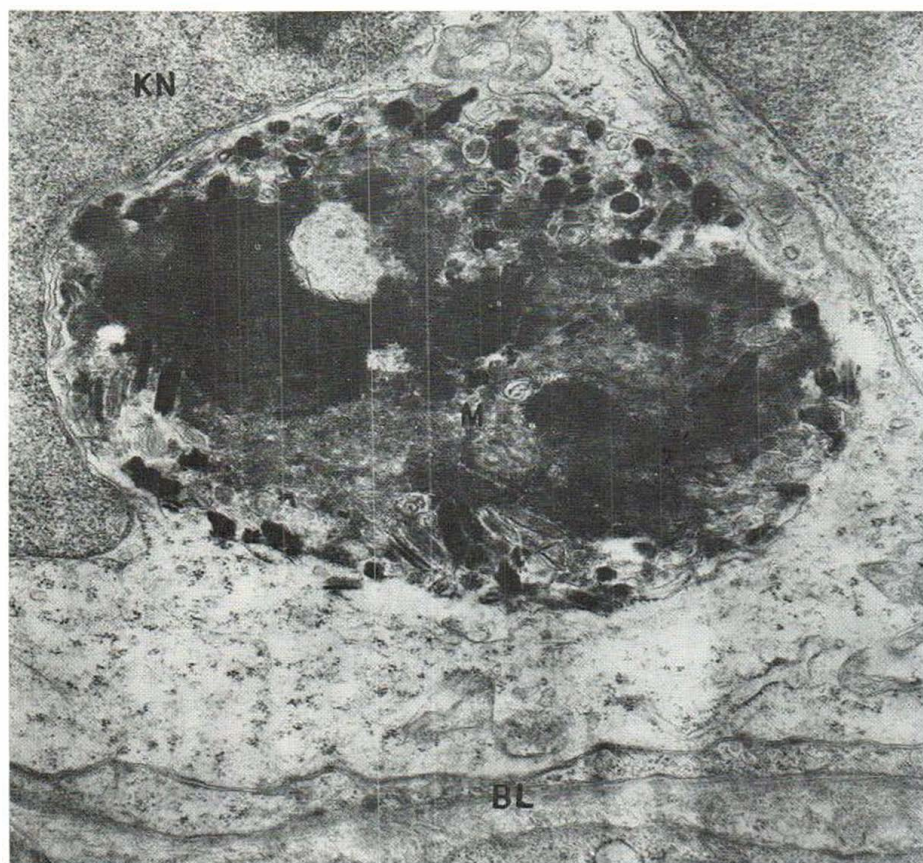


Fig. 12. This basal keratinocyte has phagocytosed a melanocyte (M). Note the numerous melanosomes as well as nuclear fragments within the phagocytosed inclusion. Day 4

postnatal mouse epidermis. B.L. = basal lamina. K.N. = basal keratinocyte nucleus. $\times 19\ 000$.

be observed in the upper stratum corneum during fetal life which also indicates that no cornified cells are shed prenatally (3). In the human, periderm persists above the developing stratum corneum until late fetal life (6, 25).

The development of the "thickened" cell membranes in the strata granulosum and corneum appears to be due to an accumulation of dense material adjacent to the cytoplasmic side of the cell membranes, although the evidence, based on the present study, is inconclusive and requires further investigation. Other investigators support this view also (4, 14, 64), though some suggest that the keratinosomal material coats the cell membranes from the outside (31). This study suggests that keratinosomes do contribute the amorphous extracellular material which is so prominent during late fetal and early postnatal life in mice, as has been suggested by others (24). This material may seal the extracellular

space and contribute to the formation of the epidermal barrier (50, 55, 63).

The manner in which the original keratohyalin granules selectively form a row near the distal cell membranes and then appear to fuse to form a continuous band of keratohyalin material with an electron-density similar to that of the individual granules is of interest. This material appears to undergo some morphological rearrangement prior to other changes presumably of a chemical nature, during the initial development of keratinized cells. Further studies of this stage of the keratinization process may be enlightening.

The presence of large amounts of intracellular glycogen appears to be a constant feature of developing mammalian epidermis. The amount of intracellular glycogen decreases first in the basal cells and then in the stratum granulosum. Glycogen persists for a longer period in the stratum spinosum.

Similar observations have been made in human fetal epidermis (6, 7, 22, 25, 51). In human fetal epidermis, glycogen is also present transiently in the extracellular space (22, 25).

The mouse epidermis approaches morphological maturity on fetal day 18. During the perinatal period, the mouse epidermis morphologically resembles that of the human adult (18, 20, 21). An epidermal plexus of nerves has been observed in the perinatal mouse epidermis (15). Intra-epidermal nerve fibers were not identifiable in the present study, nor were Merkel cells observed.

During the first few weeks of postnatal life, the mouse epidermis thins and the strata spinosum and granulosum become disorganized and incomplete (21). The stratum corneum becomes thinner. This condition persists in adulthood (52). The kinetics of the mitotic rate and rate of desquamation during this period require further study. Cellular degeneration and phagocytosis may also have a role in epidermal thinning during the perinatal period. The basal cells in particular, appear to be active in the phagocytosis of cells during this period. Degenerating cells which had not been phagocytosed were not observed. The question of cellular degeneration occurring before or after phagocytosis remains unanswered, although the present observations support the latter. Jurand (26) has noted that during the period of active regression of the mouse embryo limb ridge, cell necrosis and also phagocytosis occur. Cell degeneration occurs in many tissues during normal vertebrate development and not exclusively during regression or transformation (19).

Evidence for the origin of the melanocyte in the mouse from neural crest has been provided by the work of Rawles (38, 40). According to Rawles' studies, neural crest cells are present in the region biopsied for this study by 10½ days and these cells reach all areas of the mouse embryo on day 12. The present study suggests that either extremely few or no crest cells invade the embryonic epidermis until about the fifteenth day, which is in agreement with previous reports (44, 59, 60). This by no means precludes the possibility that numerous neural crest cells are present in the underlying dermis between 10½ and 15 days. The migrating neural crest cells cannot, however, be distinguished morphologically from other embryonic cells until their characteristic differentiation begins (2, 39). In the human embryo, neural crest cells may invade the epidermis much earlier. Non-keratinocytes are present in human

embryonic epidermis at 6 weeks, although these cells may not derive from the neural crest (6, 8).

It is unknown if neural crest cells are pluripotent initially and differentiate according to their ultimate location and the influence of local environmental factors, or if their potentialities are fixed prior to migration. The factors governing neural crest migration are unknown but in the chick at least, neural crest cells disseminated by the vascular route migrate to positions normally attained in natural development (62). It is suggested that neural crest cell may serve as a stem cell, remain latent or differentiate into a melanocyte (43). Potential melanocytes which have not undergone differentiation cannot be identified as such on a morphological basis (39). These cells can only be called indeterminate or undifferentiated dendritic cells.

The melanocytes observed between days 15 and 17 in the mouse fetus are not numerous but can be identified due to the presence of early stage melanosomes. The early melanocytes in mice and humans appear to lack cell processes (6, 60). Melanocytes in 18 and 19 day fetal mice have dendritic processes. There appears to be an increase in the number of melanocytes in mice through the third postnatal day. This increase is believed to be due primarily to the mitosis of existing melanocytes (43). At no time were melanocytes or other dendritic cells observed crossing the basal lamina.

On the fourth postnatal day, the population of epidermal melanocytes appears to be decreasing and the cells contain fewer melanosomes. Melanosomes are transferred to keratinocytes during this time. Some of the melanocytes may become inactive and indistinguishable morphologically from indeterminate cells (36, 45) although there does not appear to be a significant increase in the number of these cells during this time. Some melanocytes may migrate into hair follicles (57) and others may degenerate (41). Evidence has been presented that some melanocytes are phagocytosed by keratinocytes.

Active melanocytes can be maintained in the body of the mouse by ultraviolet light (42) or by surgical trauma (48). Amelanotic melanocytes can be stimulated to produce melanin by treatment of the skin with irritants or carcinogens (12, 27, 45, 56, 58) or by ultraviolet light (37). The number of indeterminate cells remains constant after treatment of the skin with at least one carcinogen (58). There is evidence that the melanocyte system in the mouse remains as extensive as it is in man (45, 54). The pres-

ent study supports other investigations concerning the absence of active melanocytes in the mouse 2-3 weeks after birth (36, 39, 41, 42, 48, 57).

In the 8- and 9-week-old human embryo and fetus, epidermal melanocytes have been observed with the electron microscope (17, 49). In the 11 week fetus, epidermal melanocytes can be detected by the ammoniacal silver method (66). In the 10 to 12 week fetus, melanocytes contain melanosomes in the early stages of development (32). At 12 weeks, melanocytes in the basal layer of the fetal scalp contain all stages of melanosomes (22). Between 12 and 14 weeks, basal and occasional higher melanocytes have been reported (6, 10, 67). These melanocytes are rounded and lack cell processes (6). During subsequent months, there is an increase in the number of human fetal epidermal melanocytes and dendritic processes are present (2, 34, 67).

Langerhans cells were observed only in the 18 day postnatal mouse epidermis in the present study. Langerhans cells have been reported in 6-8-day-old mice (36, 44). At 3-4 months, half of the dendritic cells are Langerhans cells and the remainder are indeterminate cells (58).

It has been suggested that the indeterminate cells may be two distinct populations, one determined to differentiate into melanocytes and the other into Langerhans cells (36). In man, it has been suggested that indeterminate cells may be premelanocytes, e-fete melanocytes, unrelated cells, or cells which may differentiate into Langerhans cells (65). In the present study, indeterminate cells are observed prior to the appearance of Langerhans cells but after the appearance of immature melanocytes.

It has been suggested that the epidermal non-keratinocytes in the 6 week human embryo may be Langerhans cell precursors (8). In the 14 week human fetus, mature Langerhans cells are present, while the melanocytes are immature and it is suggested that Langerhans cells do not arise from melanocytes (10). Langerhans cells are also capable of mitosis (28).

The problem concerning the origin of the Langerhans cell has been the subject of much experimental work. Langerhans cells have been found in grafts of neural crest-free limb buds of embryo mice grafted to adult mouse spleens but this work raised the question of potential migration of host Langerhans cells into the graft (9, 53). Reams & Tompkins (44) in more recent work grafted prospective mouse embryo skin free of neural crest into chick embryo hosts which reportedly do not have Langerhans cells.

Ultrastructural studies of the grafts revealed only indeterminate cells in younger grafts and both Langerhans cells and indeterminate cells, but no melanocytes, in older grafts. Based on this evidence, Reams & Tompkins suggest that Langerhans cells are not derived from neural crest and are not related to melanocytes but are of ectodermal origin.

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L. W. Weiss, M.D.
Department of Dermatology
University of Minnesota Hospitals
Box 98, Mayo Building
Minneapolis, Minnesota 55455
USA