Acta Dermatovener (Stockholm) 55: 321–329, 1975

EMBRYOLOGY OF THE EPIDERMIS: ULTRASTRUCTURAL ASPECTS

II. *Period of Differentiation in the Mouse with Mammalian Comparisons*

Larry W. Weiss and Alvin S. Zelickson

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

Abstract. A detailed light and electron microscopic study of the cellular morphology of the epidermis in the 13 through 16 day mouse fetus reveals that an occasional intermediate cell is interposed between the basal and peridermal layers on day 13. All layers are mitotically active. Tonofilaments, unassociated with desmosomes, are present within the basal cell cytoplasm and the mitotic axis of the basal cells has changed from a parallel to a perpendicular plane with respect to the epidermal surface. At 14 days, a complete stratum intermedium, composed of one or two cell layers, is present. Rarely, developing hemidesmosomes are observed. Pools of glycogen are present in all cells below the periderm. The periderm is dense and no longer mitotically active. The skein of filaments, present in the inferior cytoplasmic region of the basal cells on days 12 and 13, is now absent. In the 15 day fetus, numerous developing hemidesmosomes are present. The stratum intermedium contains three to four layers of cells, and filaments are located deep within the cytoplasm of these intermediate cells. Rarely, a few developing keratohyalin granules and keratinosomes are present. A stratum intermedium is no longer present in the 16 day fetus. This region is now composed of a stratum spinosum and a stratum granulosum. Numerous keratinosomes are located in the upper stratum spinosum and lower stratum granulosum. The cells in the stratum granulosum are nucleated and the uppermost cells contain large keratohyalin granules. Three heterogeneous and one homogeneous type of keratohyalin granule is described. Dense bodies are present within mitochondria, nuclei, glycogen pools and the peridermal cytoplasm. The periderm is no longer dense and glycogen and keratohyalin granules are not observed in this layer.

Key words: Embryology; Epidermis; Differentiation; Periderm; Stratum intermedium; Keratohyalin granule

This second paper in the series on the detailed morphology of the developing mammalian epidermis covers the period from the time the periderm has become established to the last day prior to the formation of the stratum corneum. In the mouse, this includes days 13 through 16 inclusive: a complete layer of periderm having been formed on day 12. During this time, and as a prerequisite for keratinization, the germinative layer gives rise to a stratum intermedium which subsequently differentiates into a lower stratum spinosum and an upper stratum granulosum.

Hanson (15) has described the light microscopic morphology of developing mouse and rat epidermis during this period. Others have also investigated the general morphology or specific aspects of the developing epidermis during portions of this stage of development in the mouse (8–10, 23, 24, 31, 32). Morphological features comparable to those in developing mouse epidermis during the period under consideration have also been observed in the rat (1, 2, 12) and human (3–5, 7, 16–19, 22, 26, 27, 33–36).

MATERIALS AND METHODS

C57B1/6 mouse fetuses, 13 through 16 days of age, were taken at 24 hour intervals and processed for light and electron microscopic studies. 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 (32).

OBSERVATIONS

13 day embryo. In addition to the basal layer and a complete layer of periderm, the developing epidermis contains occasional intermediate cells (Fig. 1). There appears to be an increase in the number of developing desmosomes. Hemidesmosomes are not present. Mitotic figures are observed in all layers. The mitotic axis of the basal cells has changed from a parallel to a perpendicular plane with respect to the epidermal surface. One daughter cell remains in the germinative layer and the other goes to the intermediate layer after mitosis. The whole epidermis is

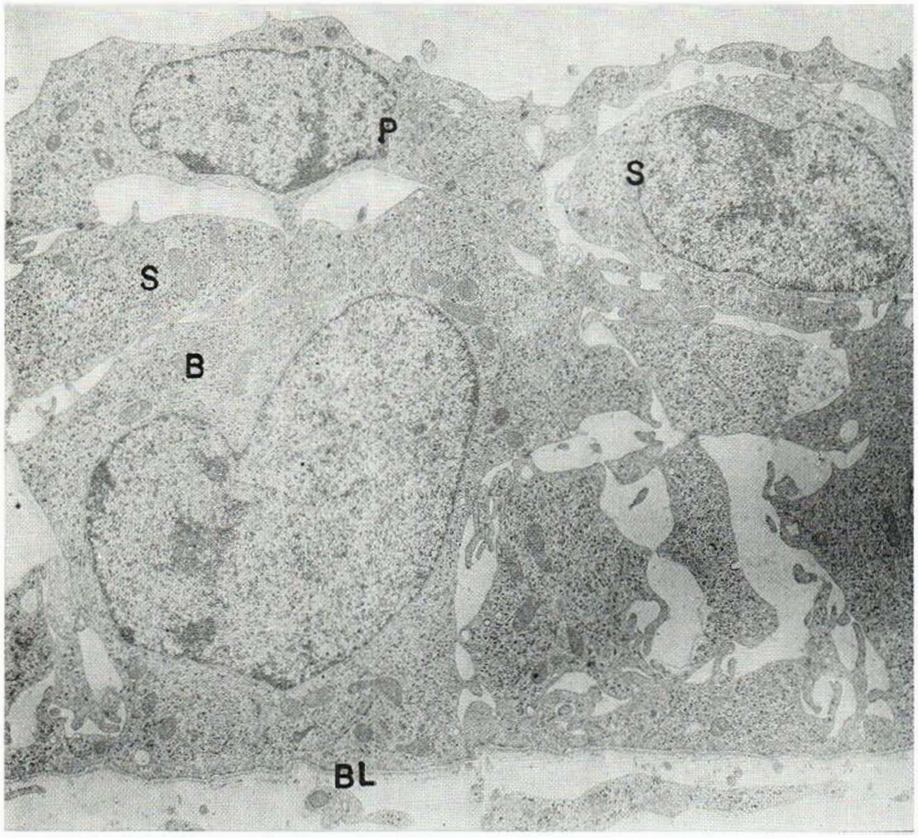

Fig. 1. Survey of the developing 13 day fetal mouse epidermis. Note the intermediate cells (*S*) between the periderm (*P*) and basal layer (*B*). *BL* = basal lamina. × 6 500.

more compact because the extracellular space has decreased in extent.

The morphology of the basal cells is similar to that of day 12. The band of filaments inside the lower basal cell membrane appears to have increased in width (Fig. 2). Many microtubules are present in non-mitotic cells. For the first time, wisps of tonofilaments appear to lie free deep within the basal cell cytoplasm (Fig. 3). The morphology of the cells in the intermediate and peridermal layers is similar to that of the basal cells. These cells lack free cytoplasmic tonofilaments but microtubules are present. The peridermal cells are flattened and have attenuated processes.

14 day embryo. There are one or two layers of cells in the stratum intermedium (Fig. 4). Both

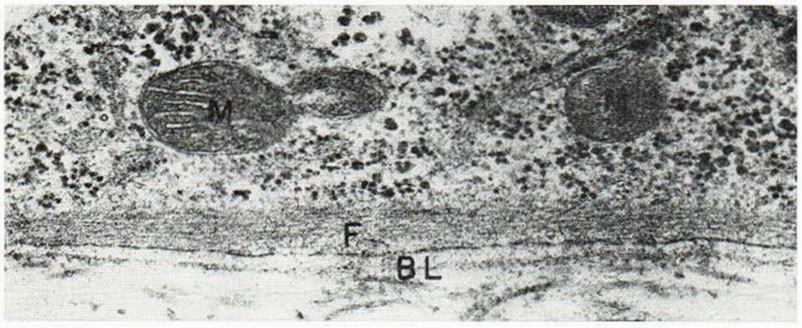

Fig. 2. A skein of filaments (*F*) is present just inside the inferior basal cell membrane of the 13 day mouse fetus. *BL* = basal lamina. *M* = mitochondria. × 37 250.

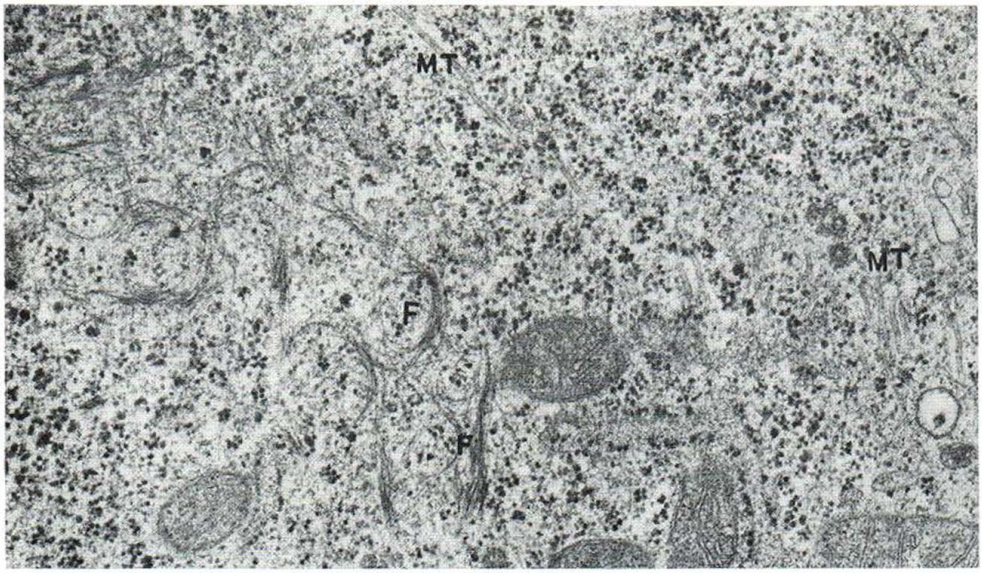

Fig. 3. Free filaments (*F*), unassociated with desmosomes and not parallel to the cell membrane, appear deep within the basal cell cytoplasm for the first time. Numerous microtubules (*MT*) are present also. 13 day mouse fetus. ×35 150.

basal and intermediate cells are mitotically active but mitotic figures are rarely observed in the periderm. The basal lamina has increased in thickness and occasionally developing hemidesmosomes are present along the inferior basal cell membranes. Developing desmosomes are most numerous in the stratum intermedium. Both light and dark basal cells, depending upon the quantity of ribosomes and glycogen, are present.

Pools of glycogen are present in the cytoplasm of all cells below the periderm (Fig. 4). Numerous mitochondria are often in close proximity to the

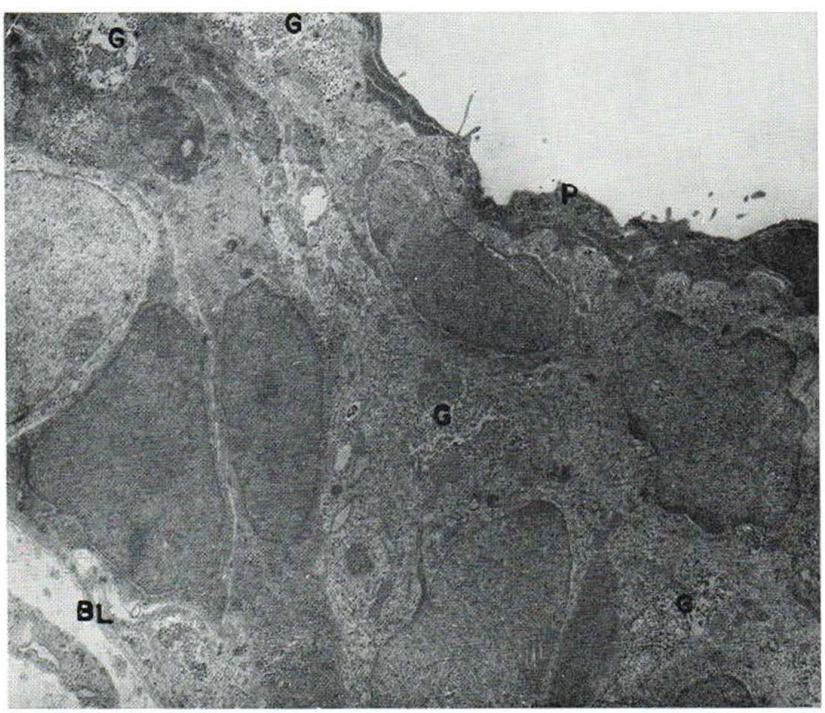

Fig. 4. Survey of the developing 14 day fetal mouse epidermis. Note the dense periderm (*P*) with few microvilli, the pools of glycogen (*G*) in the two cell layers of the stratum intermedium and in the dark basal cells. *BL* = basal lamina. ×6 850.

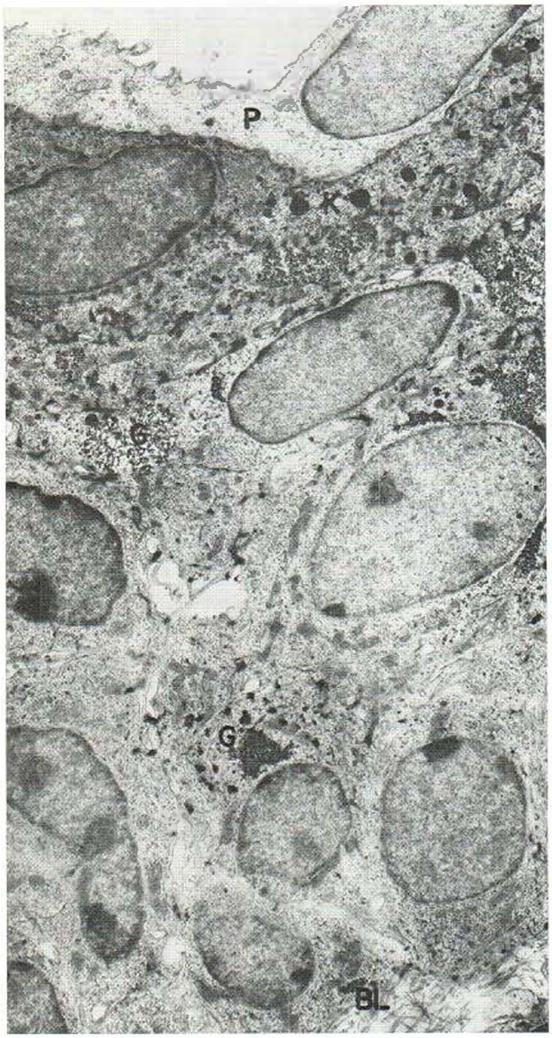

Fig. 5. Survey of the epidermis of the 16 day mouse fetal epidermis. The peridermal cells (*P*) are no longer dense. Small, dense keratohyalin granules (*K*) are present in the developing stratum granulosum. Large concentrations of glycogen (*G*) persist in all layers beneath the periderm. *BL* = basal lamina. × 4 250.

periphery of the glycogen areas. Few microtubules are observed in non-mitotic cells and the inferior layer of cytoplasmic filaments in the basal cells are not present. The elongated nuclei of the periderm are dense and no mitotic figures are present. The peridermal cytoplasm is dense too, but no glycogen is present. There are a few microvilli on the surface.

15 day embryo. The stratum intermedium contains three to four cell layers. The inferior surface of the epidermis is more irregular and numerous developing hemidesmosomes are present along the inferior basal cell membranes. Hemidesmosomes develop in a manner similar to desmosomes but are apposed by the basal lamina rather than by an adjacent cell membrane. Filaments are associated with hemidesmosomes as well as with desmosomes.

The basal and lower intermediate cells are similar in most respects. Mitotic figures and glycogen are present in both. Free cytoplasmic filaments are present for the first time in the intermediate cells. The upper portion of the stratum intermedium is not mitotically active and the cells are flattened. Rarely, a few small developing keratohyalin granules and keratinosomes are present in these cells. The Golgi

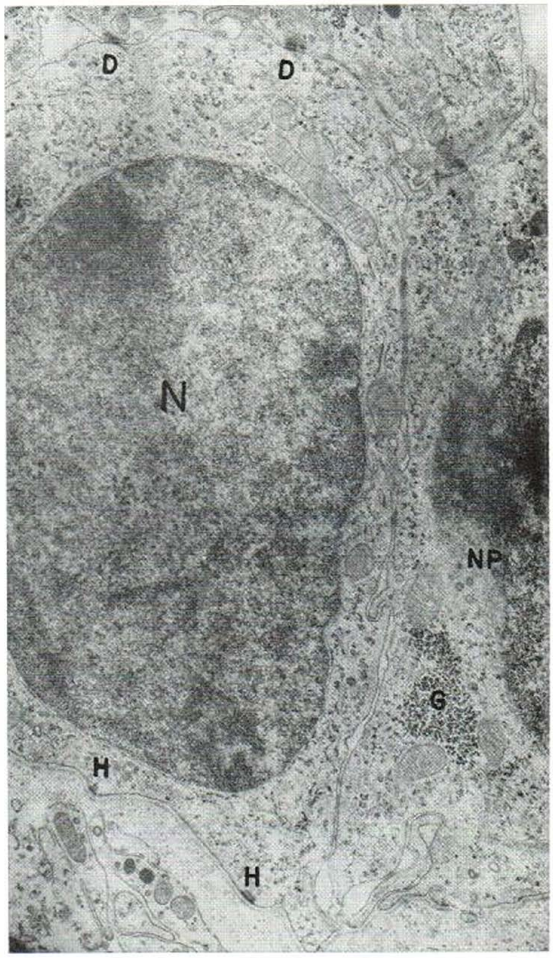

Fig. 6. Portions of two basal cells of the 16 day fetus are shown. Note the developing hemidesmosomes (*H*), glycogen (*G*), nuclear pores (*NP*) and desmosomes (*D*). *N* = portion of one basal cell nucleus. × 11 000.

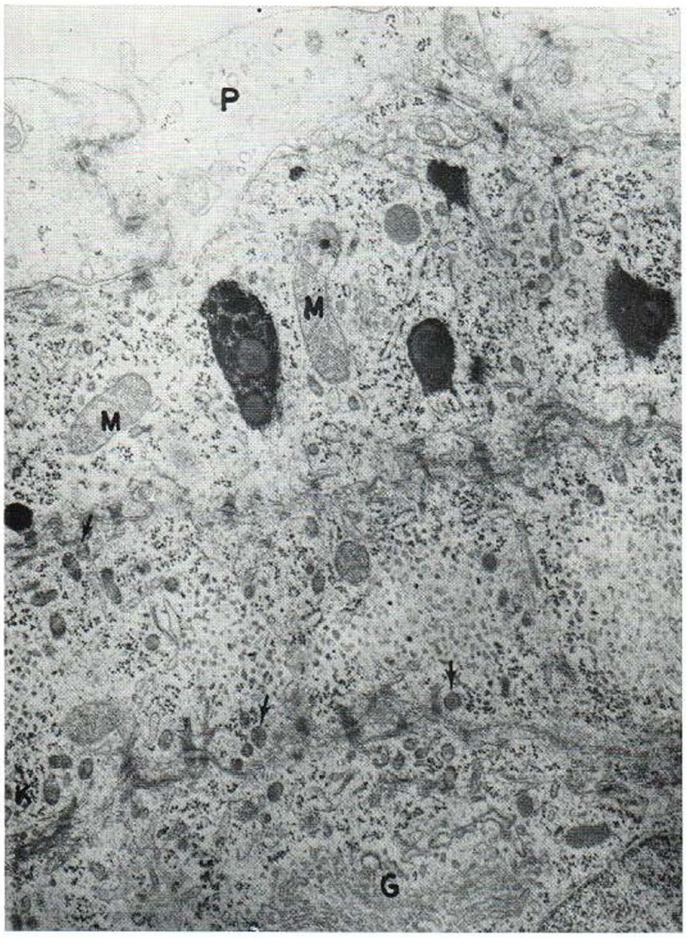

Fig. 7. Heterogeneous keratohyalin granules composed of dense particulate material and lighter oval lipoid structures are present in the granular cells just below the periderm (*P*). The heterogeneous nature of these granules is particularly evident in the keratohyalin granule located between the two mitochondria (*M*). Note the numerous keratinosomes (at arrows) and the extensive Golgi area (*G*) in the lowermost cell. 16 day mouse fetal epidermis. × 17 700.

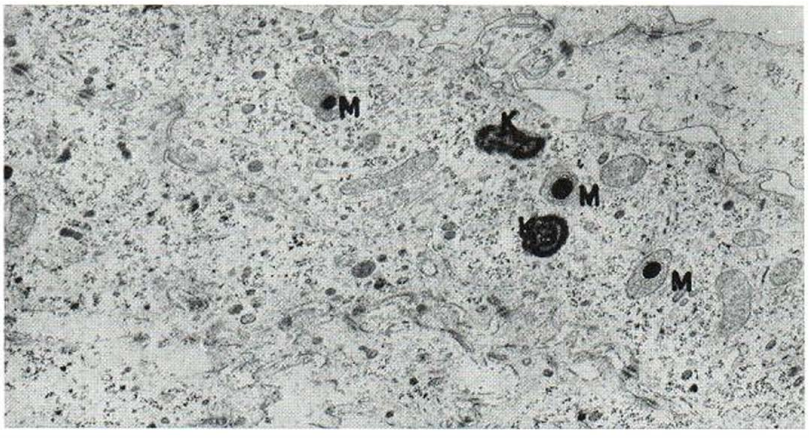

Fig. 8. The granular cell just below the periderm contains two heterogeneous keratohyalin granules (*K*) composed of finely particulate material and very electron-dense material. Three of the mitochondria (*M*) contain dense bodies which are surrounded by a unit membrane which suggests formation inside the mitochondrial cristae. 16 day mouse fetal epidermis. × 12 600.

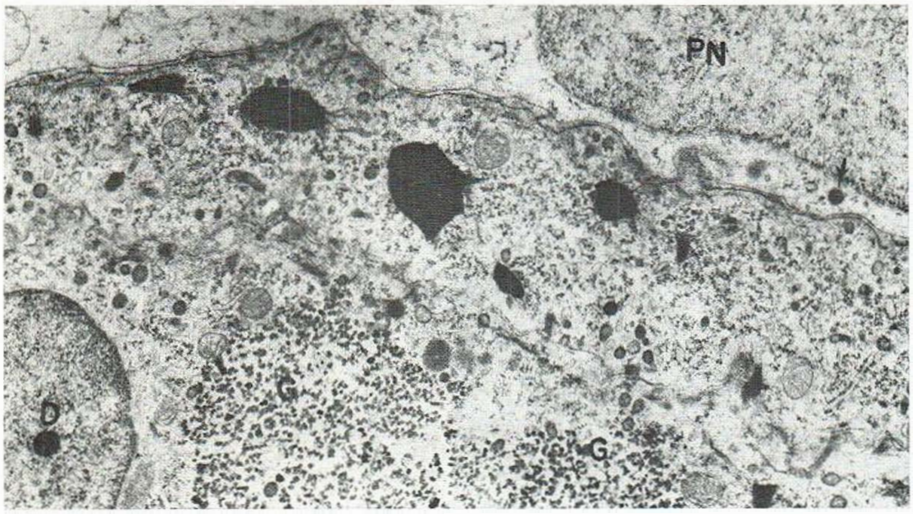

Fig. 9. A dense body (at arrow) is present in the peridermal cytoplasm just below the nucleus (*PN*) and another dense body (*D*) is located in the nucleus of a granular cell. Note the large, dense homogeneous keratohyalin granules and the concentrations of glycogen (*G*) within the stratum granulosum. 16 day fetal mouse epidermis. × 13 200.

apparatus is well developed and glycogen is present also. The peridermal cells are dense and lack centrioles, microtubules, glycogen and keratohyalin granules.

16 day embryo. A stratum intermedium is present no longer. This intermediate region now contains a lower stratum spinosum and an upper stratum granulosum (Fig. 5). The periderm is present and the stratum corneum does not form until this layer is shed. The basal lamina and hemidesmosomes are well developed (Fig. 6). Glycogen is observed in all layers below the periderm.

The stratum spinosum contains two or three cell layers and a few mitotic figures are observed in this layer. There are also two or three layers of cells in the stratum granulosum. The cytoplasm of the spiny cells contains tonofibrils which often appear to have a periodic substructure. The greatest concentration of glycogen is located in the stratum spinosum. The uppermost cells of the stratum spinosum contain numerous keratinosomes which are concentrated in the distal portions of the cell cytoplasm adjacent to the granular layer (Fig. 7). The classical lamellar substructure is observed in some of them. Keratinosomes are often located near the Golgi area also.

The cells in the stratum granulosum contain nuclei. The lowermost cells contain numerous keratinosomes and small keratohyalin granules. Tonofibrils are distributed randomly in the cytoplasm and developing keratohyalin granules are often associated with them. The uppermost granular cells contain large keratohyalin granules.

Four morphologically distinct types of keratohyalin granules are present in these cells (Fig. 7). One type of granule contains dense homogeneous material interspersed with particulate material (Fig. 8). The second type of granule contains one or two oval, moderately electron-dense areas, which may be lipoid in character, surrounded by dense homogeneous material (Fig. 7). The third type of keratohyalin granule has the combined characteristics of the first two types (Fig. 7). The fourth type of granule is composed of a homogeneous opaque material characteristic of keratohyalin granules in mature epidermis (Fig. 9). The mature type is the least abundant at this developmental stage. All keratohyalin granules appear to form in association with tonofibrils and ribosomes. The heterogeneous types appear to be formed first since they are larger and are located in the uppermost granular cells.

Some of the mitochondria in the uppermost stratum granulosum contain small, oval granules which are similar to keratohyalin granules with respect to homogeneity and opacity (Fig. 8). These granules are surrounded by a membrane which is separated from the dense body proper by a clear area. This suggests that the mitochondrial dense bodies may form inside cristae. Dense bodies are present

also in nuclei and in areas of glycogen (Fig. 9). These appear to lack a limiting membrane. Such dense homogeneous bodies are rarely observed within the the cytoplasm of the periderm also (Fig. 9). The periderm (Figs. 7–9) has lost most of its dense appearance and there is evidence of degeneration. A few vesicles, mitochondria, ribosomes and filaments are present within the cytoplasm. No glycogen or keratohyalin granules are present. The peridermal cells remain attached to each other by desmosomes.

DISCUSSION

The present observations agree with those of Hanson (15) and with Menefee's description of the 14 to 15 day epidermis (24) which corresponds to the 16 day epidermis in the present study. Brody & Larsson also described the ultrastructure of the 15- and 16-day developing mouse epidermis (8). They, however, subdivided the epidermis in a different manner, based on the ultrastructural morphology of the fibrils. They also described a five-layered attachment specialization in addition to desmosomes and they apparently did not observe the heterogeneous keratohyalin granules which are present in the 16 day fetus.

The period of rapid differentiation occurs from the thirteenth through the sixteenth fetal day. On day 12, only a bi-laminar epidermis is present but by day 17 cornified cells are observed. In the 13 day embryonic epidermis, cells in all layers are mitotically active but thereafter no mitotic figures are observed in the periderm. The stratum intermedium begins to form during this time and these intermediate cells are mitotically active. No mitoses are observed in the stratum granulosum after it develops. In humans, the stratum intermedium forms at about the tenth fetal week (7, 27), and one to three layers of cells are present by the twelfth week (16). It is during this period of development that the total epidermal mitotic activity is at its peak. In the rat, the mitotic activity also reaches a peak just before differentiation and decreases as a cornified layer is formed (30). Keratinization in the rat occurs between the seventeenth and twenty-first day of fetal life and, as maturation occurs, mitotic activity becomes restricted to the basal layer (1). Cell division must occur prior to differentiation and as the epidermal cells become differentiated, the ability to divide seems to be lost.

It is interesting to note that the basal cells change their plane of division just prior to the beginning of hemidesmosome formation and that the period of rapid cell proliferation and differentiation occurs during this time. This change in the plane of cleavage of basal cells in the embryonic mouse epidermis, from the beginning of stratification to the beginning of cornification, has been reported previously (29).

A few immature hemidesmosomes are present at 14 days in the fetal mouse and at 7 weeks in the human embryo (22). At 14 days, intracellular glycogen is present in all layers except the periderm in the mouse. In the human embryo, glycogen is present in both basal and peridermal cells during the second month (4, 6, 22, 35). Glycogen is observed in the human and mouse stratum intermedium (8, 22). The periderm of the mouse during this period appears to be active in absorption and then becomes dense and subsequently degenerates. The periderm of humans is more active and appears to have secretory and glycogenous functions also (3–5, 7, 16–17, 33–36).

Keratohyalin formation begins in the 15 day mouse fetus and the stratum granulosum forms a complete layer by day 16. The heterogeneous keratohyalin granules, observed on day 16 and containing lipoid and particulate material, have been observed in embryonic mice (9) and rats (2). The various types of heterogeneous granules appear simultaneously in the mouse but in the rat they appear at successively older ages and are present in the stratum corneum at birth (2). Studies of dense homogeneous deposits in keratohyalin granules indicate that these are not artifacts but the density varies with the fixative employed (14). In the human fetus, keratohyalin granules appear in the thicker regions of the epidermis at the end of the fifth month and in the sixth month the granules are present in other body regions (27). The heterogeneous types of keratohyalin granules have not been reported in man.

Keratinosome formation begins on day 15 in the mouse fetus. In man, keratinosomes have been reported in the cells of the 14 to 16 week human fetus (16). Keratinosomes are located near the Golgi area when they are first formed (21, 25). In the 15 to 16 day mouse fetal epidermis, keratinosomes fused with the cell membranes or located in the extracellular space are not observed.

Dense bodies are present in some mitochondria and nuclei in the 16 day fetal mouse epidermis. In the mitochondria, the granules are surrounded by a membrane which is separated from the dense homogeneous body by a clear area. This suggests that the

intramitochondrial dense bodies form inside cristae (31). Dense bodies in other locations lack a limiting membrane. These dense bodies have been observed in embryonic and postnatal mice (2, 8, 13, 20, 28) and in the rat (11). There is no evidence that the dense bodies attain a large size or disrupt the mitochondrial membranes. The presence of dense bodies in the periderm cytoplasm on rare occasions has not been previously reported to the best of the authors' knowledge.

ACKNOWLEDGEMENTS

This study was supported by the Anatomical Sciences Training Grant No. GM-114, U.S.P.H.S. and by Research Training in Dermatology, Grant No. 5T01-AM-05560, U.S.P.H.S. Westwood Pharmaceutical Inc. kindly provided a grant to cover the cost of publication of the electron photomicrographs.

REFERENCES

1. Bauer, F. W. & Kuyper, Ch. M. A.: Differentiation and keratinization of fetal rat skin. Dermatologica *144:* 332, 1972.
2. Bonneville, M. A.: Observations on epidermal differentiation in the fetal rat. Am J Anat *123:* 147, 1968.
3. — The periderm of human fetal skin. *In* Proceedings of the Third European Regional Conference of Electron Microscopy, 1964 (ed. M. Tillback), p. 565. Royal Microscopical Society, Prague, 1965.
4. Breathnach, A. S.: An Atlas of the Ultrastructure of Human Skin. 398 pp. J. & A. Churchill, London, 1971.
5. — Embryology of human skin. A review of ulstrastructural studies. J Invest Dermatol *57:* 133, 1971.
6. Breathnach, A. S. & Robins, J.: Ultrastructural features of epidermis of a 14 mm (6 weeks) human embryo. Br J Dermatol *81:* 504, 1969.
7. Breathnach, A. S. & Wyllie, L. M.: Fine structure of cells forming the surface layer of the epidermis in human fetuses at fourteen and twelve weeks. J Invest Dermatol *45:* 179, 1965.
8. Brody, I. & Larsson, K. S.: Morphology of mammalian skin: Embryonic development of the epidermis sublayers. *In* Biology of the Skin and Hair Growth (ed. A. G. Lyne & B. F. Short), pp. 267–290. American Elsevier, New York, 1965.
9. DuBrul, E. F.: The fine structure of the differentiating mouse epidermis. Am Zool *9:* 1126, 1969.
10. — Fine structure of epidermal differentiation in the mouse. J Exp Zool *181:* 145, 1972.
11. Farbman, A. I.: Morphological variability of keratohyalin. Anat Rec *154:* 275, 1966.
12. Fraser, D. A.: The development of the skin of the back of the albino rat until the eruption of the first hairs. Anat Rec *28:* 203, 1928.
13. Frei, J. V. & Sheldon, H.: Corpus intra-cristam: A dense body within mitochondria of cells in hyperplastic mouse epidermis. J Biophys Biochem Cytol *11:* 724, 1961.
14. Fukuyama, K., Wier, K. A. & Epstein, W. L.: Dense homogeneous deposits of keratohyalin granules in newborn rat epidermis. J Ultrastruct Res *38:* 16, 1972.
15. Hanson, J.: The histogenesis of the epidermis in the rat and mouse. J Anat *81:* 174, 1947.
16. Hashimoto, K., Gross, B. G., DiBella, R. J. & Lever, W. F.: The ultrastructure of the skin of human embryos. IV. The epidermis. J Invest Dermatol *47:* 317, 1966.
17. Hoyes, A. D.: Acid mucopolysaccharide in human fetal epidermis. J Invest Dermatol *48:* 598, 1967.
18. — Electron microscopy of the surface layer (periderm) of human fetal skin. J Anat *103:* 321, 1968.
19. Kobayasi, T.: Development of fibrillar structures in human fetal skin. An electron microscope study. Acta Morphol Neerl-Scand *6:* 257, 1966.
20. Matoltsy, A. G. & Parakkal, P. F.: Keratinization. *In* Ultrastructure of Normal and Abnormal Skin (ed. A. S. Zelickson), p. 76. Lea & Febiger, Philadelphia, 1967.
21. Matoltsy, A. G. & Parakkal, P. F.: Membrane-coating granules of keratinizing epithelia. J Cell Biol *24:* 297, 1965.
22. Matsunaka, M. & Mishima, Y.: Electron microscopy of embryonic human epidermis at seven and ten weeks. Acta Dermatovener (Stockholm) *49:* 241, 1969.
23. Menefee, M. G.: The differentiation of keratin-containing cells in the epidermis of embryo mice. Anat Rec *122:* 181, 1955.
24. — Some fine structure changes occurring in the epidermis of embryo mice during differentiation. J Ultrastruct Res *1:* 49, 1957.
25. Parakkal, P. F.: An electron microscopic study of esophageal epithelium in the newborn and adult mouse. Am J Anat *121:* 175, 1967.
26. Selby, C. C.: An electron microscope study of the epidermis of mammalian skin in thin sections. I. Dermoepidermal junction and basal cell layers. J Biophys Biochem Cytol *1:* 429, 1955.
27. Serri, F., Montagna, W. & Mescon, H.: Studies on the skin of the fetus and child. II. Glycogen and amylophosphorylase in the skin of the fetus. J Invest Dermatol *39:* 199, 1962.
28. Setala, K. L., Merenmies, L., Stjernvall, L. & Nyholm, M.: Mechanism of experimental tumorigenesis. IV. Ultrastructure of interfollicular epidermis of normal adult mouse. J Natl Cancer Inst *24:* 329, 1960.
29. Smart, I. H. M.: Variation in the plane of cell cleavage during the process of stratification in the mouse epidermis. Brit J Dermatol *82:* 276, 1970.
30. Stein, I. B., Dayton, L. & Duecy, J.: The uptake of tritiated thymidine by the dorsal epidermis of the fetal and newborn rat. Anat Rec *170:* 225, 1971.
31. Weiss, L. W.: A Light and Electron Microscopic Study of the Development of the Epidermis in the Mouse, 303 pp. Ph.D. Dissertation, University of Minnesota, 1970.
32. Weiss, L. W. & Zelickson, A. S.: Embryology of the epidermis: Ultrastructural aspects. I. Formation and early development in the mouse with mammalian comparisons. Acta Dermatovener (Stockholm) *55:* 161, 1975.
33. Wolf, J.: Curve of development and disappearance of peridermal phase of human embryonic skin demon-

strated by the replica method. Folia Morph (Praha) *16:* 36, 1968.

34. Wolf, J.: Structure and function of periderm. I. Superficial structure of the peridermal epithelium. Folia Morph (Praha) *15:* 296, 1967.
35. — Structure and function of periderm. II. Inner structure of peridermal cells. Folia Morph (Praha) *15:* 306, 1967.
36. — The replacement of periderm by epidermis. Folia Morph (Praha) *16:* 24, 1968.

Received December 2, 1974

L. Weiss, M.D.
Department of Dermatology
University of Minnesota Hospital
Box 98
Minneapolis, Minnesota 55455
USA