

EMBRYOLOGY OF THE EPIDERMIS: ULTRASTRUCTURAL ASPECTS

I. Formation and Early Development in the Mouse with Mammalian Comparisons

Larry W. Weiss and Alvin S. Zelikson

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 developing epidermis in the 7 to 12 day mouse embryo reveals that a single layer of ectoderm is not present until day 8. The 8-day embryonic epithelium is characterized by the presence of microvilli, apical attachment specializations, developing desmosomes and a thin, immature basal lamina. The 9-day embryonic epithelium is flatter and has both an increased number of microvilli on the surface and an increased number of microtubules in association with developing desmosomes. On day 10, numerous developing desmosomes are associated with fine filaments in the regions of the attachment plaques. Occasional peridermal cells are present on day 11. Numerous granules are associated with the inferior cell membranes of the superficial or uppermost cell processes. In the 12-day embryo, the periderm forms a complete layer. A skein of fine filaments is present just inside the basal cell membrane of the germinative cells and microtubules are more abundant in the basal cell cytoplasm.

Key words: Embryology; Epidermis; Keratinocytes; Periderm; Developing epidermis

There have been many investigations of short periods of epidermal development as well as observations on certain of its specific aspects. With the recent increased availability of human tissue, our knowledge of the morphology of developing human epidermis has increased (7). Light microscopic observations of the rat and mouse, spanning the period from early epidermal development through the postnatal period, have been reported (14, 18). Bonneville (3) has also reported on the ultrastructural aspects of the periderm, keratohyalin granules and the keratinization process in fetal rats, and DuBrul (12) has studied ultrastructural differentiation in the mouse.

The present study was undertaken to provide a detailed account of the general morphology, at the ultrastructural level, of the formation, subsequent development, and maturation of the mammalian

epidermis. This study is intended to provide a detailed account of the whole embryological and early postnatal spectrum of all morphological aspects of epidermal development. Due to the difficulty in obtaining human material of known gestational age and at regular time intervals, the mouse was chosen for this investigation. Correlations between electron and light microscopic observations in the mouse will be made as well as comparisons with published ultrastructural studies of human epidermal development. This report covers the 7-12 day gestation period of the mouse. Subsequent papers in this series will deal with progressively more advanced stages of epidermal development.

MATERIAL AND METHODS

Timed matings of virgin C57Bl/6 female mice, obtained from the Jackson Laboratories, Bar Harbor, ME, were used in this investigation. The day of mating was considered as day zero in the determination of gestational age. Embryos were taken at 24 hour intervals beginning with day 7. The embryonic age is accurate to within ± 2 hours.

Pregnant females of the desired gestational age were sacrificed by cervical dislocation, the whole uteri were removed and placed in cold saline. Each uterine segment which contained a 7-day embryo was isolated and these embryos were fixed in utero. Embryos 8 to 12 days of age were dissected free of uterine tissue and membranes under a binocular dissecting microscope. Embryos from each litter were then divided randomly into 2 groups: one for electron microscopic studies and one for light microscopy. Skin was removed from the mid-dorsolateral region of the animals. A minimum of 6 embryos from a minimum of 3 litters were used for each age group and for each of the 2 respective groups.

For light microscopy, the embryos were fixed in Bouin's fixative, processed routinely and stained with haematoxylin and eosin. For electron microscopy, only 8-12 day embryos were studied. These were fixed for 2 hours in cold buffered 1% osmium tetroxide, dehydrated in alcohol and embedded

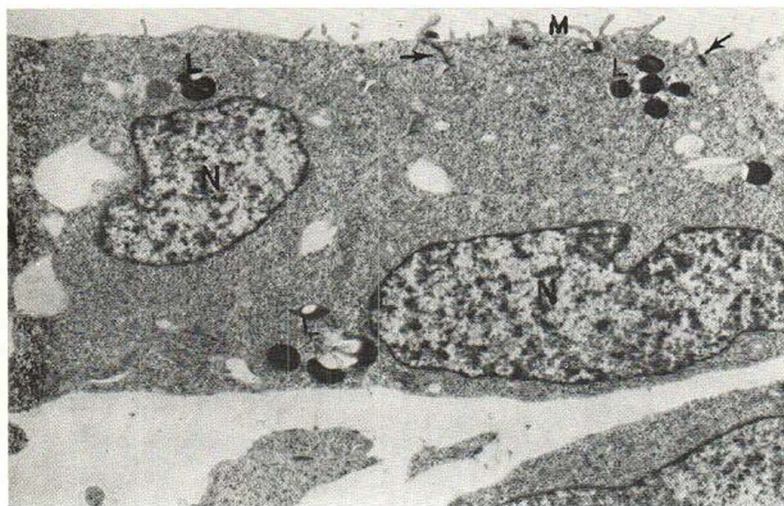


Fig. 1. Survey of the 8-day mouse embryonic epithelium. Note the single layer of cells, microvilli (*M*), developing apical attachment specializations (*arrows*) and opaque lipid inclusions (*L*). *N* = nucleus. $\times 5\,000$.

in Epon (25). Thin sections were cut on an LKB Ultratome, stained with uranyl acetate (34) followed by lead citrate (30) and viewed with an RCA-3G electron microscope operated at 100 kV.

OBSERVATIONS

7- and 8-day embryos. The 7-day embryo is cylindrical. The ectoderm is unorganized and forms a thick inner mass of cells. A single layer of ectodermal epithelium is absent.

A simple cuboidal layer of ectodermal epithelium covers the dorsum of the 8-day embryo (Fig. 1) between the neural plate and the amniotic epithelium. The cell membranes are in close proximity primarily at the proximal and distal lateral cell borders. A few immature attachment specializations, characterized by the close proximity of the cell membranes and the presence of adjacent homogeneous intracellular electron-dense material, are present at the apico-lateral cell boundaries (Fig. 2).

A few developing desmosomes are present and are usually located between the upper, outer portions of two cells. During desmosome development, both the adjacent cell membranes and the leaflets of the individual membranes become evenly spaced. The spacing between the outer leaflets of the two cell membranes is wider than that between the inner and outer leaflets of each membrane respectively. The outer and then the inner leaflets seem to become increasingly opaque. Electron-dense material is present on the cytoplasmic side of the inner leaflets. Less electron-dense material is present in the extracellular space of this region. An intercellular contact



Fig. 2. Apical attachment specializations (*arrow*) between adjoining cells in the 8-day mouse embryo are characterized by the close proximity of the cell membranes and the presence of dense, homogeneous material as shown. *M* = microvilli. $\times 22\,200$.

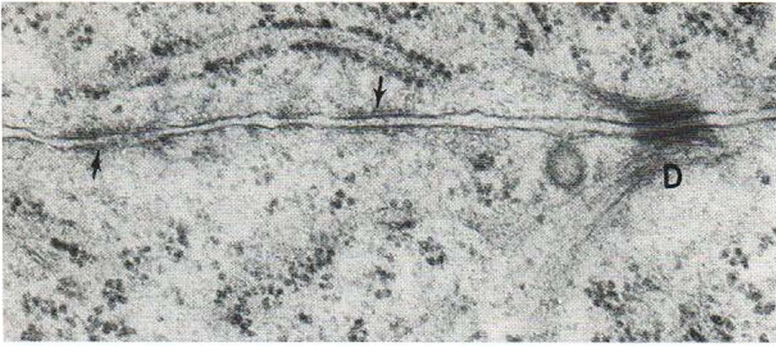


Fig. 3. Developing desmosomes (arrows) are commonly observed and rarely a more mature desmosome (at D), containing an intercellular contact layer and associated tonofilaments, is present. Eight-day mouse embryo. $\times 54\,700$.

layer develops, characterized initially by a series of small particles which later appear to become confluent to form the characteristic dense line. The leaflets of the cell membrane subsequently increase in opacity and more electron-dense material is deposited in the cytoplasm adjacent to the inner leaflets. Very rarely, the more mature desmosomes are associated with tonofilaments (Fig. 3). These sweep in from the cytoplasm, closely parallel the attachment plaque, and then sweep out into the cytoplasm again. Microtubules are sometimes present near the desmosomes. Hemidesmosomes are not present.

The basal lamina, composed of a thin, moderately opaque band of filamentous material, closely follows the smooth contour of the cells. Microvilli are present at the cell surface. A few fine filaments are sometimes visible within the microvilli. The nuclei are large, are located in the basal portion of the cytoplasm, and contain one or two nucleoli. The cytoplasm is filled with clusters of free ribosomes. The endoplasmic reticulum is not extensive, contains an amorphous material within its cisternae, and has contiguous rough and smooth areas along the membranes. Ribosomes are also associated with areas of the outer nuclear membrane. The Golgi apparatus is well-developed and is usually located in a supranuclear position. Oval and elongate mitochondria, containing a granular matrix, are scattered randomly in the cytoplasm.

Centrioles are present and the cells divide in a plane parallel to the surface of the ectoderm. Desmosomes remain intact during the mitotic process. Microtubules are present in non-mitotic cells. They are located near the cell membranes and are often clustered near developing desmosomes. A few vesicles containing an amorphous material are present near the basal aspects of the cells. Larger, very opaque lipid inclusions are often seen.

9-day embryo. The ectoderm of this stage is similar morphologically in many respects to that of day 8 but a few changes are noteworthy. The epithelium is more flattened and there is more intercellular space. A greater number of developing desmosomes



Fig. 4. Survey of the junctional area between two epithelial cells and their developing attachment specializations (arrows). The more developed desmosomes are located toward the outer portions of the cells. M= microvilli, B=developing basal lamina. $\times 26\,800$.

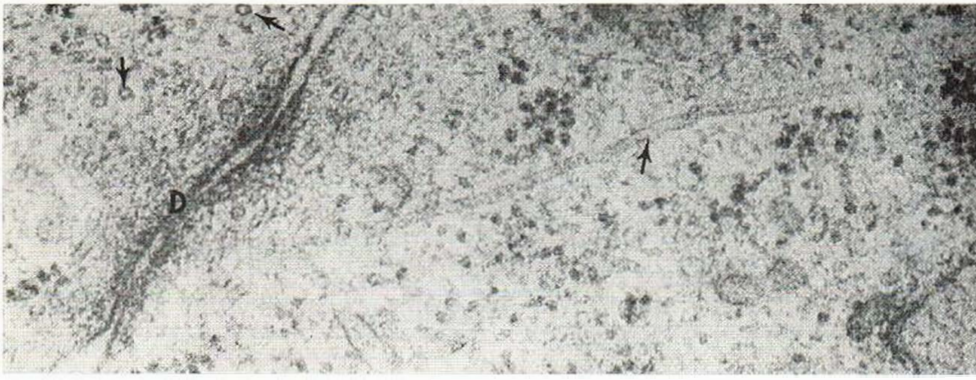


Fig. 5. Developing desmosomes in the 9-day mouse embryo are frequently juxtaposed by microtubules. Microtubules (arrows) are oriented both parallel and perpendicular to the

desmosomes and have an outer diameter of approximately 200 Å. $\times 83\ 300$.

are present (Fig. 4) and more microtubules are present in juxtaposition to the desmosomes (Fig. 5). The microtubules are oriented both parallel and perpendicular to the long axis of the desmosomes and some of them extend far into the cytoplasm. Other microtubules are in juxtaposition to apical junctional attachment specializations (Fig. 6).

More microvilli are present. The basal lamina is very thin and no basement membrane is visible by light microscopy. Mitotic cells are observed frequently. The Golgi apparatus is more extensive and better developed. There are fewer lipid inclusions and most of them have lost much of their electron opacity.

10-day embryo. The cells are less flattened and less extracellular space is present. There are fewer

microvilli. The apical attachment specializations are well developed. Numerous developing desmosomes are associated with tonofilaments. Hemidesmosomes are absent. The basal lamina has increased slightly in width. Fewer microtubules and lipid inclusions are present. The Golgi apparatus appears to be very active. Numerous vesicles are present in the apical cytoplasm near the Golgi apparatus.

11-day embryo. Occasional periderm cells are present above the basal layer of the developing epidermis. These cells are very flattened and have long cytoplasmic extensions overlapping a number of basal cells (Fig. 7). Apical attachment specializations are present between peridermal cells or between periderm and basal cells also. Filamentous

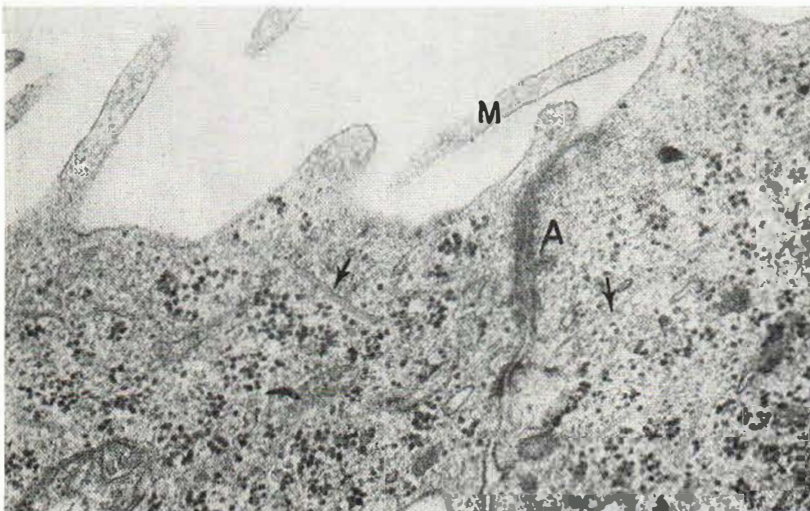


Fig. 6. Microtubules (arrows) are present near developing apical attachment specializations (A). M = microvilli. $\times 49\ 200$.

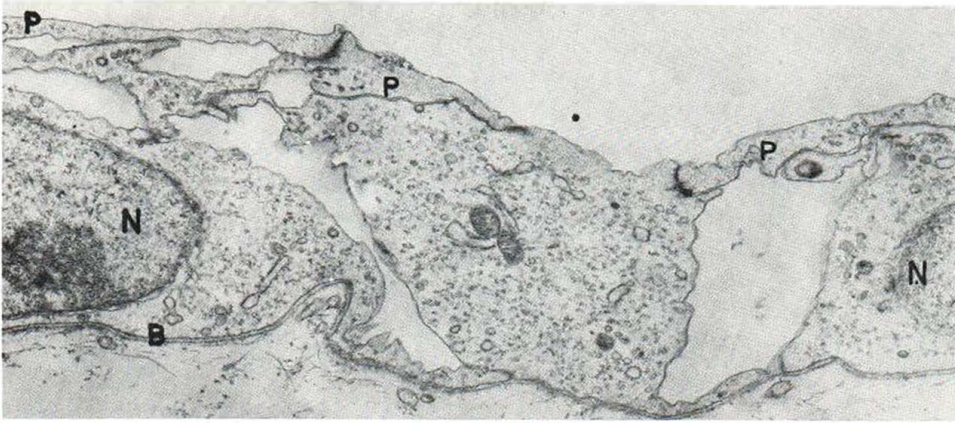


Fig. 7. Survey of the 11-day embryonic mouse epithelium. Note the numerous cell processes. Occasional peridermal

cells are present at this age. *B* = basal lamina; *N* = nucleus; *P* = peridermal cell process. $\times 10\,300$.

material is associated with the desmosomes and few microtubules are present. Both the basal and peridermal cells are mitotically active. Long rows of membrane-limited granules which contain an amorphous, electron-dense material are often located in the basal region of the superficial cell processes (Fig. 8). None of these dense granules were noted to have fused with the cell membrane. A few fine filaments are also present in the superficial processes.

12-day embryo. The developing epidermis is now a fully stratified epithelium which contains a complete upper layer of periderm and a basal layer. The long axes of the basal cells are oriented perpendicular to the surface of the epidermis. There are numerous cell processes between the cells and a substantial amount of extracellular space is present. The developing desmosomes in the upper portion are more developed than those between the lower portions of the basal cells. Some of the basal cells have a series of short processes which project into the dermis (Fig. 9). A dense skein of filaments is present just inside the lower basal cell membrane in a number of cells. These filaments extend across the full width of the cell (Fig. 10). Microtubules

are present just above this band of filaments. Microtubules again are plentiful throughout the basal cell cytoplasm (Fig. 11). Rows of vesicles containing opaque material are no longer present in the superficial cell processes. The periderm also contains a few fine filaments and microtubules. Both cell layers are mitotically active and have a similar content and distribution of typical cell organelles.

DISCUSSION

The present observations on epidermal development in the mouse are in agreement with those of other investigators, although minor differences occur in the correlations of morphology with embryonic age (12, 18). These differences are probably due to different methods utilized in the determination of age and to the different strains of mice used. The morphology of developing mouse epidermis also correlates well with early developmental stages in the rat (14, 18) and human (6, 7).

Reinius (29) found that no presumptive epidermal ectoderm is present in the 7½-day mouse embryo. In the 8-day embryo, a simple layer of ectoderm is present on the dorsal trunk (1, 35–37). The establish-

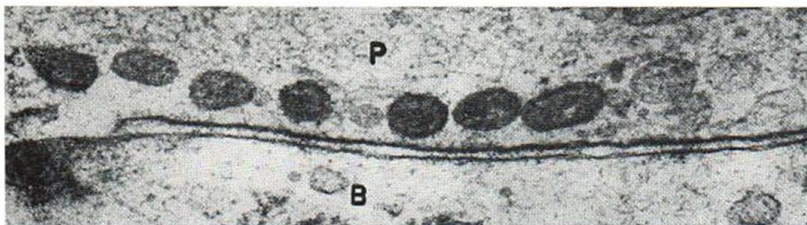


Fig. 8. Membrane-limited granules, 1 to 2 μm in diameter, are often present in rows along the inferior aspect of the superficial peridermal cell processes (*P*) of the 11-day mouse embryo. *B* = basal cell process. $\times 61\,350$.



Fig. 9. Short basal cell processes (at BP) extend toward the dermis. The basal lamina (arrows) closely approximates the irregular contour of the basal cells. A portion of a mitotic cell (M) is shown on the right. Twelve-day mouse embryo. $\times 10\,050$.

ment of the surface epithelium follows an orderly sequence of morphological events (26): cell adherence, microvilli formation, attachment specialization development, basement membrane formation, and formation of intracellular filaments.

Attachment specializations form first at the apicolateral cell borders. These apical specializations are not desmosomes but the techniques employed here do not demonstrate clearly whether these are classical tight junctions (13) or gap junctions (15, 16, 43). Desmosomes seem to develop first between the upper portions of the cells. There is general agreement concerning the morphological events characterizing desmosome formation in amphibians (26), birds (27) and mammals (7, 35, 37). The material forming the desmosomal attachment plaques seems to differ chemically from that of the cell membrane and intercellular contact layer (11, 24, 44). The factors involved in determining the site of desmosome formation are unsolved. In the chick embryo, unpaired desmosomes between two cells are incapable of inducing the formation of their counterparts in opposing cells (27).

The basal lamina appears to be formed very early

in development. There is convincing morphological evidence that developing chick corneal epithelium forms its own basal lamina (17, 32, 33). Immunohistochemical evidence also suggests that many basement membranes in the mouse embryo are formed by the overlying epithelial tissue (28). Recent work with epidermal-dermal recombinants and grafts shows convincingly that adult human skin also forms its own basal lamina (10). There is no good morphological evidence of the source of the basal lamina in the developing mouse or human embryonic (6) epidermis.

Filaments are the last structures to appear in association with developing desmosomes (6, 7, 26, 27, 35, 37). Few filaments are present during the early stages of epidermal development. The microtubules noted in the present study have been observed previously in embryonic epidermis (5, 35-37). No correlation between the presence and orientation of microtubules and the change in cell shape is noted in this study. The association of microtubules with developing desmosomes suggests that microtubules may have a role in maintaining cell shape. The microtubules which are located in the

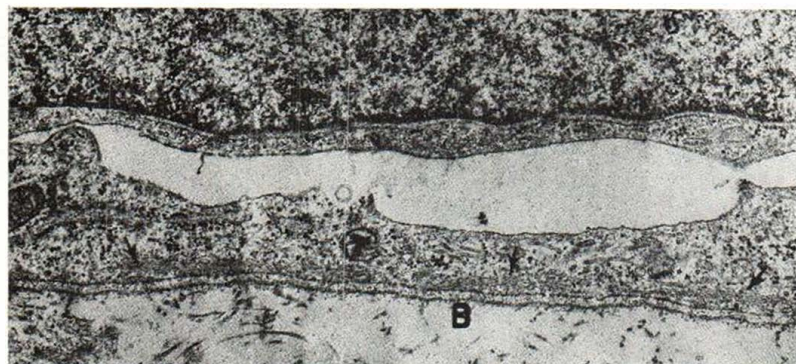


Fig. 10. A narrow skein of filaments (arrows) is located just inside the inferior basal cell membrane and microtubules are present just above the filaments. The basal lamina (B) is about 400 Å wide. $\times 19\,600$.

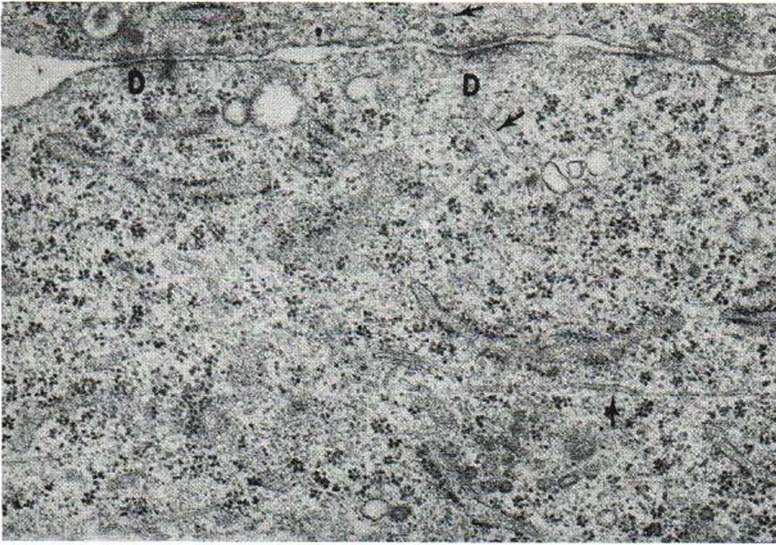


Fig. 11. Developing desmosomes (D) are present between the basal and peridermal cells. Note microtubules (arrows), randomly located in the cytoplasm, and vesicles. Twelve-day mouse embryo. $\times 24\,900$.

basal region of the germinative layer of the 12-day embryo have a more orderly arrangement and may, together with the basal skein of filaments, provide some support. The basal cells must support the overlying periderm and they still lack the stabilization provided by hemidesmosomes. The basal layer of filaments has been observed in the basal cells of chick embryos (23), in cultured basal cells (38), in embryonic mice (22) and in human (6–8) basal cells.

The periderm forms a complete layer in the 12-day mouse embryonic epidermis. The presence of microvilli, seen on the surface first of the ectoderm and then of the periderm, suggests that the periderm has an absorptive function in addition to its protective role. There is no clear evidence of a secretory function in either the mouse or the rat (3). In the human, the periderm appears to have both a secretory and a glycogenous function (4, 6, 7, 9, 19–21, 39–42). In the mouse, the periderm lacks glycogen deposits. In the human, the periderm is formed in the fourth embryonic week of development (31). The developing epidermis of human (31) and monkey (2) during approximately the first month of embryonic life is similar to that of the 8 to 9 day mouse embryonic epidermis.

ACKNOWLEDGEMENTS

This study was supported by Anatomical Sciences Training Grant No. GM-114, USPHS, and by Research Training in Dermatology, Grant No. 5T01-AM-05560, USPHS. Westwood Pharmaceuticals, Inc., kindly provided a grant to cover publication costs.

REFERENCES

1. Allen, E. & MacDowell, E. C.: Variation in mouse embryos of 8 days gestation. *Anat Rec* 77: 165, 1940.
2. Bell, M.: Ultrastructure of differentiating epidermis in fetal rhesus monkeys. *Anat Rec* 160: 312, 1968.
3. Bonneville, M. A.: Observations on epidermal differentiation in the fetal rat. *Am J Anat* 123: 147, 1968.
4. — 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.
5. Branson, R. J.: Orthogonal arrays of microtubules in flattening cells of the epidermis. *Anat Rec* 160: 109, 1968.
6. Breathnach, A. S.: An Atlas of the Ultrastructure of Human Skin, 398 pp. J. & A. Churchill, London, 1971.
7. — Embryology of human skin. A review of ultrastructural studies. *J Invest Dermatol* 57: 133, 1971.
8. Breathnach, A. S. & Robins, J.: Ultrastructural features of epidermis of a 14 mm. (6 weeks) human embryo. *Br J Dermatol* 81: 504, 1969.
9. Breathnach, A. S. & Wyllic, 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, 1975.
10. Briggaman, R. A., Dalldorf, F. G. & Wheeler, C. E.: Formation and origin of the basal lamina and anchoring fibrils in adult human skin. *J Cell Biol* 61: 384, 1971.
11. Douglas, W. H. J., Ripley, R. C. & Ellis, R. A.: Enzymatic digestion of desmosomes and hemidesmosome plaques performed on ultrathin sections. *J Cell Biol* 44: 211, 1970.
12. Dubrul, E. F.: Fine structure of epidermal differentiation in the mouse. *J Exp Zool* 181: 145, 1972.
13. Farquhar, M. G. & Palade, G. F.: Cell junctions in amphibian skin. *J Cell Biol* 26: 263, 1965.
14. Fraser, D. A.: The development of the skin of the back of the albino rat until the eruption of the first hairs. *Anat Rec* 38: 203, 1928.

15. Goodenough, D. A. & Revel, J. P.: A fine structural analysis of intercellular junctions in the mouse liver. *J Cell Biol* 45: 272, 1970.
16. — Formation of pentalaminar structures by treatment of "gap" junctions with acetone. *J Cell Biol* 43: 44a, 1969.
17. Goodfellow, R. A., Revel, J. P. & Hay, E. D.: Secretion of collagenous connective tissue by corneal epithelium. *Anat Rec* 163: 191, 1969.
18. Hanson, J.: The histogenesis of the epidermis in the rat and mouse. *J Anat* 81: 174, 1947.
19. 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.
20. Hoyes, A. D.: Acid mucopolysaccharide in human fetal epidermis. *J Invest Dermatol* 48: 598, 1967.
21. — Electron microscopy of the surface layer (periderm) of human fetal skin. *J Anat* 103: 321, 1968.
22. Jurand, A.: Ultrastructural aspects of early development of the forelimb buds in the chick and mouse. *Proc Royal Soc London, B* 162: 387, 1965.
23. Kallman, F., Evans, J. & Wessells, N. K.: Normal epidermal basal cell behavior in the absence of basement membrane. *J Cell Biol* 32: 231, 1967.
24. Komura, J. & Ofuji, S.: Effect of electron stains and desmosome: An electron microscopic study. *J Invest Dermatol* 48: 304, 1967.
25. Luft, J. H.: Improvements in epoxy resin embedding methods. *J Biophys Biochem Cytol* 9: 409, 1961.
26. Mercer, E. H.: Protein synthesis and epidermal differentiation. In *The Epidermis*, (ed. W. Montagna & W. C. Lobitz), p. 161. Academic Press, New York, 1964.
27. Overton, J.: Desmosome development in normal and reassociating cells in the early chick blastoderm. *Devel Biol* 4: 532, 1962.
28. Pierce, G. B.: The development of basement membranes of the mouse embryo. *Devel Biol* 13: 231, 1966.
29. Reinius, S.: Morphology of the mouse embryo from the time of implantation to mesoderm formation. *Z Zellforsch Mikrosk Anat* 68: 711, 1965.
30. Reynolds, E. S.: The use of lead citrate at high pH as an electronopaque stain in electron microscopy. *J Cell Biol* 17: 208, 1963.
31. Serri, F., Montagna, W. & Mescon, H.: Studies of the skin of the fetus and child. II. Glycogen and amylophosphorylase in the skin of the fetus. *J Invest Dermatol* 39: 199, 1962.
32. Trelstad, R. L.: The Golgi apparatus in chick corneal epithelium: Changes in intracellular position during development. *J Cell Biol* 45: 34, 1970.
33. — Vacuoles in the embryonic chick corneal epithelium, an epithelium which produces collagen. *J Cell Biol* 48: 689, 1971.
34. Watson, M. L.: Staining of tissue sections for electron microscopy with heavy metals. *J Biophys Biochem Cytol* 4: 475, 1958.
35. Weiss, L. W.: A Light and Electron Microscopic Study of the Development of the Epidermis in the Mouse, 303 pp. Ph.D. Diss., University of Minnesota, 1970.
36. Weiss, L. W. & Zelickson, A. S.: The basal region of developing epidermis: An ultrastructural study. *J Invest Dermatol* 54: 346, 1970.
37. Weiss, L. W., Zelickson, A. S. & Heggstad, C. B.: Membrane modifications in developing embryonic epidermis: An electron microscopic study. *Anat Rec* 163: 288, 1969.
38. Wessells, N. K.: Differentiation of epidermis and epidermal derivatives. *New Engl J Med* 277: 21, 1967.
39. Wolf, J.: Curve of development and disappearance of peridermal phase of human embryonic skin demonstrated by the replica method. *Folia Morph (Praha)* 16: 36, 1968.
40. — Structure and function of periderm. I. Superficial structure of the peridermal epithelium. *Folia Morph (Praha)* 15: 296, 1967.
41. — Structure and function of periderm. II. Inner structure of peridermal cells. *Folia Morph (Praha)* 15: 306, 1967.
42. — The replacement of periderm by epidermis. *Folia Morph (Praha)* 16: 24, 1968.
43. Wolff, K. & Schreiner, E.: An electron microscopic study on the extraneous coat of keratinocytes and the intercellular space of the epidermis. *J Invest Dermatol* 54: 37, 1968.
44. — Differential enzymatic digestion of cytoplasmic components of keratinocytes: Electron microscopic observations. *J Ultrastruct Res* 36: 437, 1971.

Received August 19, 1974

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