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Variation in Basement Membrane Topography in Human Thick Skin

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ABSTRACT Samples of human plantar and palmar skin were excised and incubated in 20 mM EDTA after which the epidermis was gently separated from the dermis with the plane of separation occurring in the lamina lucida. Scanning electron microscopic examination of the dermal component revealed the classically described series of regularly spaced grooves and papillae that characterize the epidermal-dermal junction in thick skin. Primary dermal grooves exhibited evenly spaced tunnels that were originally occupied by sweat gland ducts. The basement membrane (basal lamina) in the primary grooves was relatively smooth but did exhibit a flattened, reticulated pattern at high magnifications. The basement membrane of secondary dermal grooves and papillae was in the form of numerous, elevated microridges off of which septae arose at roughly right angles. The surface appearance of the basement membrane in these areas was that of a honeycomb owing to the numerous compartments and recesses formed by the ridges and septae. Degradation of the basement membrane by trypsin demonstrated that the foundation for the highly folded and compartmentalized basement membrane was composed of dermal collagen fibrils, 60–70 nm in diameter, that were arranged in a series of variably sized, interconnected collagen bundles or walls. Epidermal basal cells extended cytoplasmic (foot) processes into two or more compartments, formed by the ridges and septae, which considerably amplified the basement membrane surface available for epidermal attachment. Scanning electron microscopic studies of the epidermal-dermal junction confirm the variable surface character of this interface previously reported by others using sectioned material. This regional variation in surface architecture apparently distinguishes between areas in which epidermal basal cells are specialized for attachment (papillae, secondary dermal grooves) and regions occupied by slow cycling epidermal stem cells from which mitotically active keratinocytes arise.

A complex epidermal-dermal junction forms in human thick skin during the middle and late stages of fetal development (Okajima, 1975). The dermal half of this junction consists of a series of primary and secondary grooves that are separated by rows of dermal papillae.[1] Corresponding epidermal ridges fill the dermal grooves and the nature of the epidermal-dermal fit establishes the unique patterns of ridges and grooves (dermatoglyphs) that are present on the surface of the palmar and plantar epidermis (Cummins and Mildo, 1943; Penrose, 1968). The structure of the epidermal-dermal junction in both thick (Misumi and Akiyoshi, 1984) and thin skin (Hull and Warfel, 1983) has been the subject of recent scanning electron microscopic (SEM) studies. In this paper, we add to these recent studies by presenting our SEM observations on the location-dependent, variable surface topography of the epidermal basement membrane in thick skin. We also clarify the surface appearance of the basement membrane as opposed to that of the dermal collagen fibrils immediately subjacent to the basement membrane.

MATERIALS AND METHODS

Skin samples from eight human cadavers, ranging from 50 to 84 years old, were studied. Samples of skin approximately 5 × 5 mm, free of subcutaneous connective tissue, were removed from the ball and arch of the foot, thenar eminence of the hand, and the distal palmar surface of the thumb. Two additional samples of plantar skin that measured 1.5 × 1.5 cm were also studied. The epidermis was removed from the dermis essentially as described by Scaletta and MacCallum (1972). Briefly, skin samples were incubated for 90–120 minutes at 37°C

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[1] In this paper the terms primary and secondary dermal grooves as proposed by Mulvihill and Smith (1969) are used. This terminology reflects the sequence of embryologic development of these structures. Secondary dermal grooves are also termed dermal furrows (Penrose, 1968).

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in a pH 7.2, balanced salt solution (BSS) that contained 20 mM EDTA. The epidermis was then gently lifted off the dermis using fine forceps. This technique results in epidermal-dermal separations in the plane of the lamina lucida and leaves the basal lamina (lamina densa) intact, affixed to the dermis (Scaletta and MacCallum, 1972).[2] To partially degrade the basal lamina, selected dermal samples were further incubated in 0.25% crude (1:250) trypsin in BSS for 2 hours at 37°C. This procedure results in the partial to complete dissolution of the basal lamina as described by Scaletta and MacCallum (1974).

Following epidermal separation or enzyme treatment, the dermal samples were pinned, epidermal surface up, to a wax surface to minimize curling or other dimensional changes during fixation. Fixation was accomplished by adding cacodylate-buffered 2.5% glutaraldehyde to the BSS that covered the tissue. The BSS-glutaraldehyde mixture was gradually withdrawn and replaced with full strength 2.5% glutaraldehyde and fixed at room temperature for 2 hours. This procedure minimizes clumping or partial collapse of dermal papillae. Some specimens were fixed further with cacodylate-buffered 1% OsO_4 for 2 hours at 4°C, but this procedure did not improve the SEM appearance of the tissue. Specimens were critical point dried in CO_2 and coated with gold-palladium before viewing.

RESULTS

The observations presented are intended primarily to describe the regional variations in surface topography of the epidermal basement membrane rather than to illustrate the differences in the arrangement of dermal grooves and papillae which are responsible for the distinctive dermatoglyphic patterns found on the foot, palm, or thumb. The topography of the basement membrane, as described below, is constant in the areas studied and appears to be independent of the arrangement and curvature of dermal grooves or age-related changes in the structure of dermal papillae. Most of the photomicrographs presented in the paper are of the plantar surface of the foot where the relatively parallel course of the dermal grooves facilitated flat and tilted SEM observations of the basement membrane.

The complex of dermal grooves and papillae can easily be resolved even at very low magnifications. Occasional bifurcations or blind endings of secondary dermal grooves (furrows), and the palisades of papillae that surround them, are also evident (Fig. 1). At higher magnifications, the primary dermal grooves exhibit regularly spaced tunnels that extend deep into the dermis (Fig. 2). These tunnels normally contain the ducts of eccrine sweat glands which remain attached to the epidermis when it is separated from the dermis. The secondary dermal groove lacks any interruptions and is usually narrower than the primary groove. The two grooves are separated by dermal papillae that occur in a wide variety of conformations. A common arrangement is that of from three to five individual papillae arising from a common base (Fig. 2).

When the surface of the primary dermal groove is viewed at higher magnification, it exhibits a distinctly reticulated appearance somewhat like a series of nets, each having a different hole size, piled on top of one another (Figs. 3, 5). The surfaces of the papillae and secondary dermal grooves are characterized by a series of microridges that run roughly parallel to one another (Figs. 3, 4). In general, the microridges are oriented parallel to the long axis of the dermal grooves or papillae. Some papillae have a slightly spiral arrangement of the microridges that, regardless of their orientation on the deeper aspects of the papillae, usually terminate as broad sinuous folds on the papillary tips (Fig. 4).

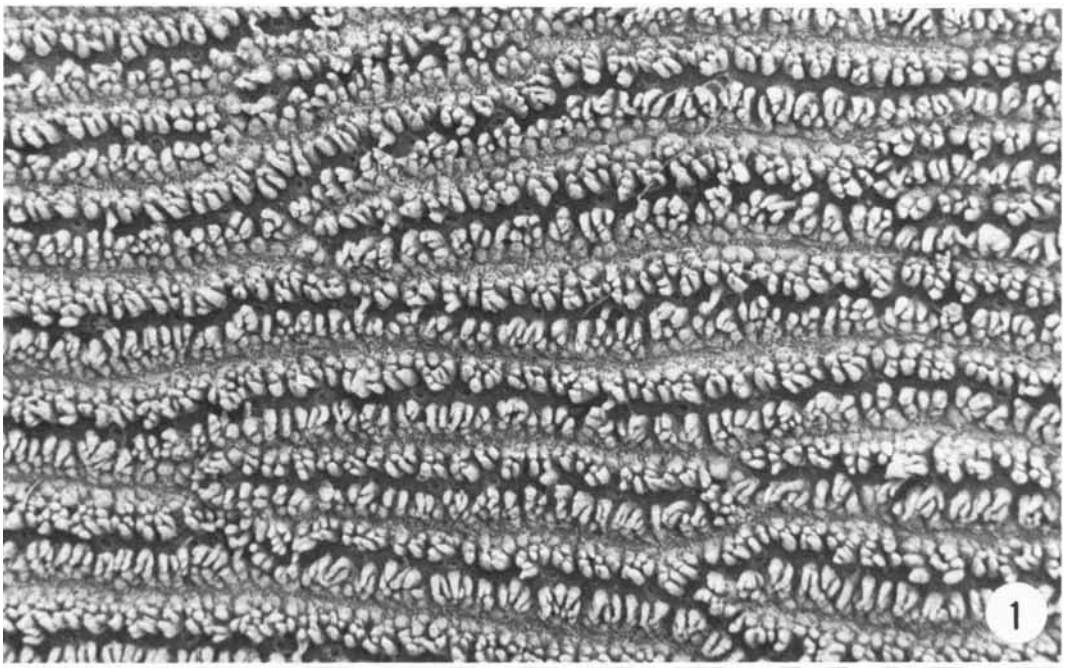
Epidermal separation was often incomplete following EDTA incubation of large specimens ($>1 \times 1$ cm), and, as a consequence, numerous solitary basal cells remained attached to the lower aspects of the dermal papillae and the secondary grooves. This fortuitous and unanticipated occurrence afforded a view of basal cell relationships to the highly folded basement membrane (Figs. 6, 7, 8). Basal cell cytoplasmic (foot) processes are placed into several different recesses or compartments formed in the plicated surface (Figs. 6, 7). Presumably, the space remaining in each compartment would be filled by the foot processes of adjacent basal cells. When viewed from directly above at high magnification, the surface of the basement membrane exhibited the previously described microridges off of which numerous microseptae originate at roughly right angles. The resulting honeycombed surface is covered by a basal lamina that is smooth in surface view with virtually no discernible substructure (Fig. 8).

Trypsin degradation of the basal lamina does not alter the general arrangement of the dermal surface. It does, however, unmask the underlying dermal collagen fibrils (Figs. 9–11). On papillae and secondary grooves, support for the complexly folded basal lamina is provided by aggregates of dermal collagen fibrils, 60–70 nm diameter, that branch to form the honeycomblike foundation upon which the basement membrane is deposited. The organization of collagen in the primary dermal grooves is less complex and consists of a netlike arrangement of collagen fibrils. Occasional, partially degraded fragments of the basal lamina could be observed applied to the underlying collagen fibrils following trypsin treatment (Fig. 12).

DISCUSSION

Recent studies by Hull and Warfel (1983) and Misumi and Akiyoshi (1984) have demonstrated that a complex folded pattern characterizes the dermal surface of the epidermal-dermal junction, a pattern that cannot easily be observed without the resolution afforded by scanning electron microscopy. Our findings agree with many of the observations made by Misumi and Akiyoshi in their study of the relationship between the form of the dermal component of the epidermal-dermal junction and the shape of the overlying fingerprint. In addition to confirming many of their findings, we can also clarify some of their observations and shall, therefore, comment more extensively on their findings than is customary. Misumi and Akiyoshi refer to the microridges depicted in the

[2]Terminology regarding the "basement membrane" is becoming increasingly confusing as more investigators work on this structure and as more becomes known regarding the function and distribution of the various macromolecules that comprise it. In this paper the terms lamina lucida and basal lamina (lamina densa) are used where precision is required (see Scaletta and MacCallum, 1972). Where such precision is not required, the less precise, but more widely understood term, basement membrane, is employed.



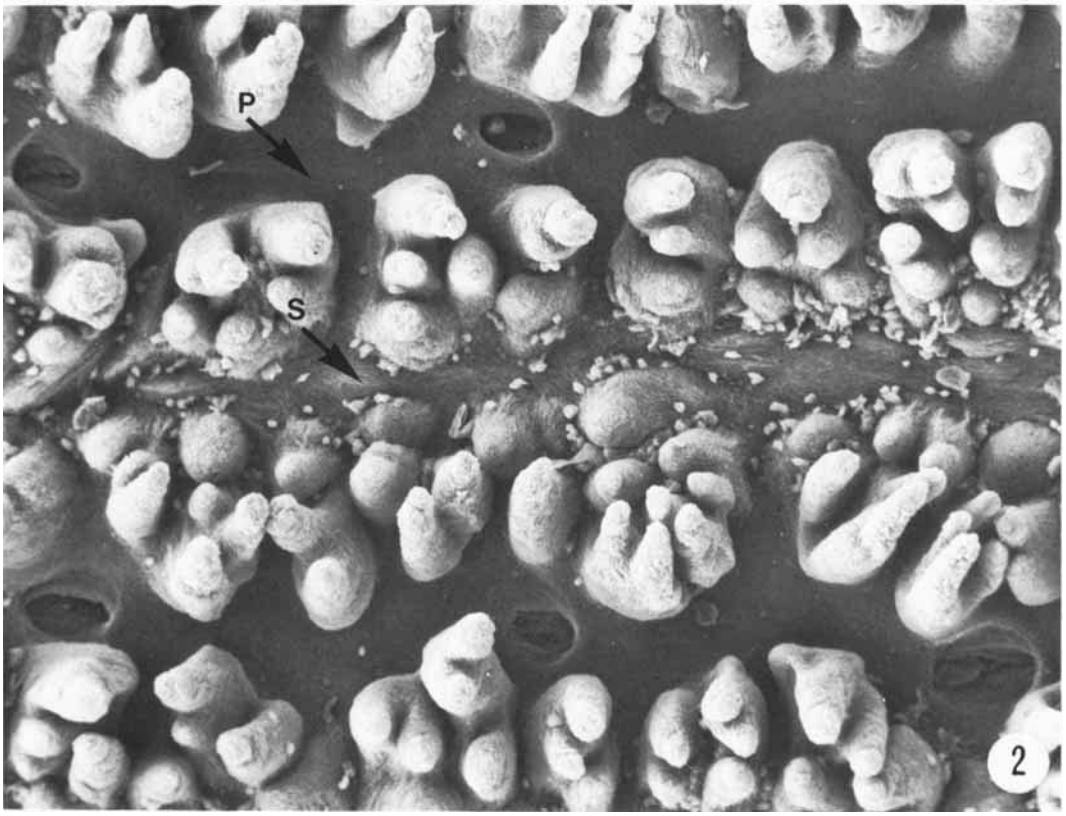


Fig. 1. Low power SEM view of the plantar dermis following removal of the epidermis. Occasional bifurcations of secondary dermal grooves (furrows) are evident as are the rows of dermal papillae that separate the primary and secondary grooves (see Fig. 2). × 22.

Fig. 2. SEM of primary (P) and secondary (S) dermal grooves. The primary grooves exhibit regularly spaced tunnels that originally contained sweat gland ducts. Dermal papillae are arranged in clusters with three or more papillae arising from a common base. The small, particlelike objects in the secondary grooves are epidermal basal cells that remained attached to the basement membrane following separation of the epidermis. × 130.

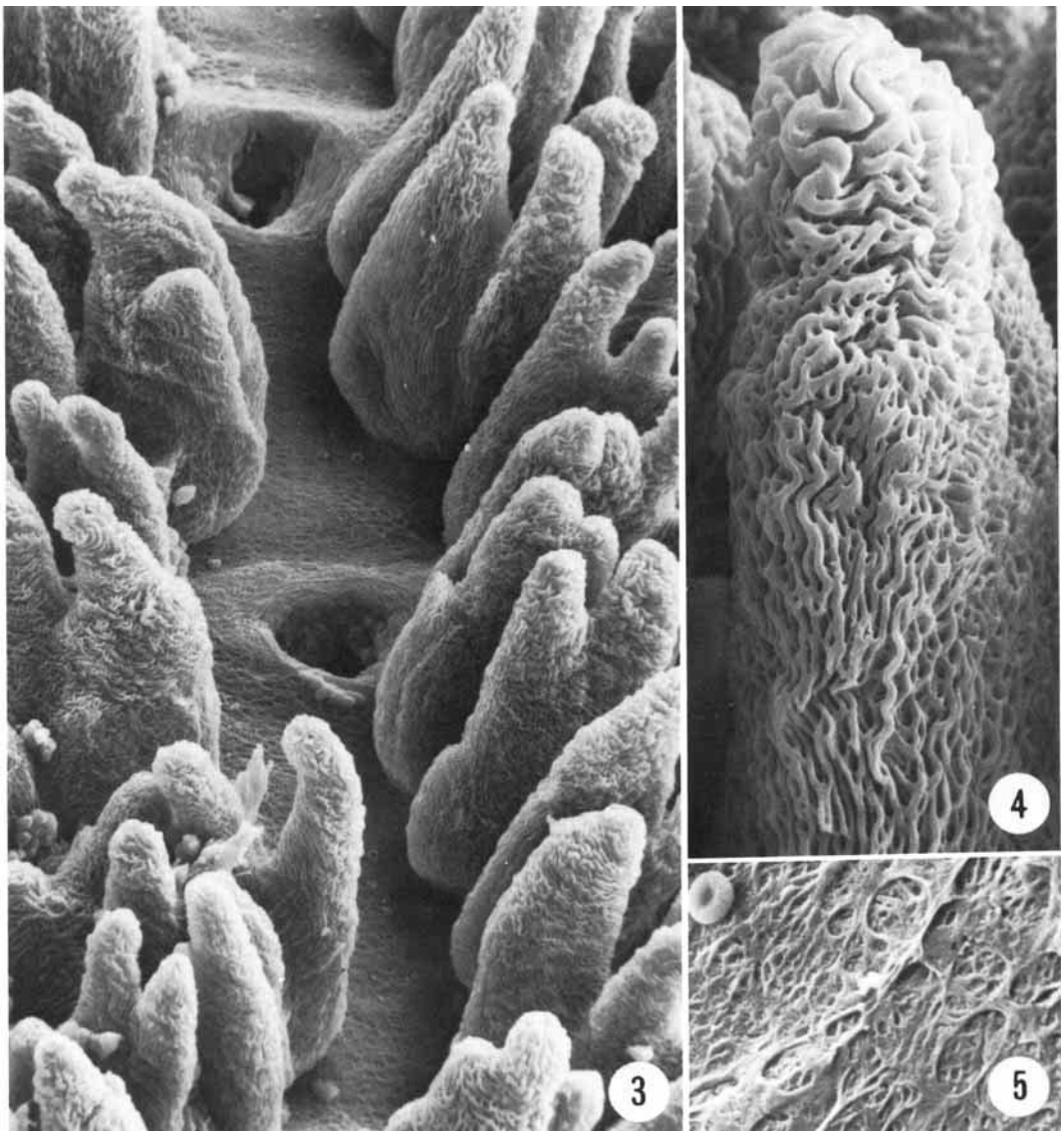


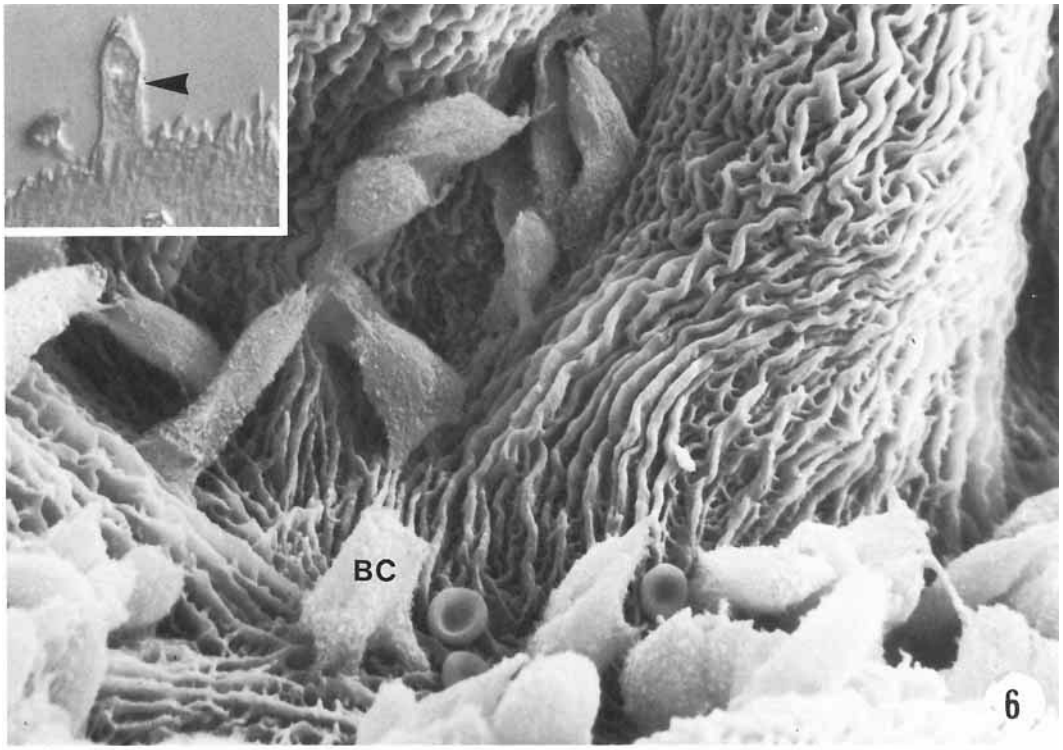
Fig. 3. SEM that illustrates the location-dependent differences in surface texture of the basement membrane. In primary dermal grooves the basement membrane has a reticulated appearance (see Fig. 5) whereas the membrane on dermal papillae exhibits a highly plicated surface (see Fig. 4). × 260.

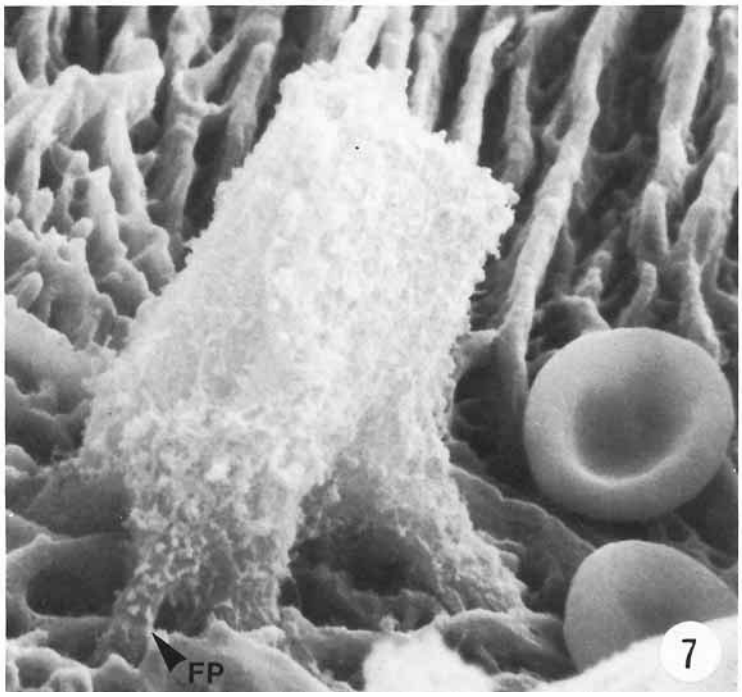
Fig. 4. The basement membrane on the deeper aspects of dermal papillae is arranged in a series of roughly parallel microridges and valleys. The ridges terminate as broad sinuous folds, as illustrated in this micrograph, on most papillary tips. ×1,040.

Fig. 5. The irregular, reticulated appearance of the basement membrane found in primary dermal grooves is illustrated. A red blood cell is present in the upper left corner of the micrograph. ×1,230.

present study as "fibers." The orientation of "fibers" described by these investigators generally agrees with the orientation of microridges observed in our study, i.e., parallel structures that are oriented along the long axis of a secondary groove and that usually assume a more spiral or "meshlike" (honeycomb) form on papillae. In addition, we agree that papillary tips frequently exhibit broad, sinuous folds or "undulations." Misumi and Akiyoshi describe the floor of the primary groove as "smooth" which is accurate in a relative sense when compared with the surfaces of secondary grooves or papillae. In fact, the surface of the primary dermal groove exhibits a distinct reticulated pattern and is not smooth in a literal sense.

By using an epidermal separation technique (EDTA chelation) that predictably and reliably results in an intact basal lamina remaining affixed to the dermis (Scaletta and MacCallum, 1972) and then subsequently removing the basal lamina by proteolytic digestion (Scaletta and MacCallum, 1974), we have clarified the surface appearance of the basal lamina (gold-palidium is deposited directly on the basal lamina; the lamina





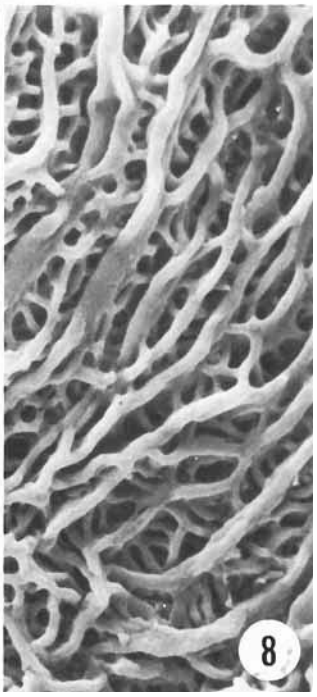


Fig. 6. Solitary basal cells present in the secondary dermal groove and the bases of dermal papillae are illustrated. The floor of the secondary grooves exhibits the same degree of basement membrane folding as do the papillae. The interrelationship of the basal cell (BC) with the folded basement membrane is illustrated in Figure 7. ×1,300. Inset, solitary basal cell (arrowhead), similar to those illustrated in the SEM, in a secondary dermal groove. Note the serrated dermal surface. One-micrometer thick section. Normarski interference optics. ×950.

Fig. 7. Epidermal basal cells extend foot processes (FP) into several different compartments or recesses formed by the folding of the basement membrane. In intact epidermis, foot process of two or more basal cells probably share the same recess or compartment. The illustrated red blood cells provde a convenient size reference. ×4,700.

Fig. 8. Small septae originating at roughly right angles from microridges are evident when the basement membrane surface is viewed from directly above. The resulting honeycomb pattern considerably amplifies the surface of the basement membrane available for basal cell attachment. ×2,800.

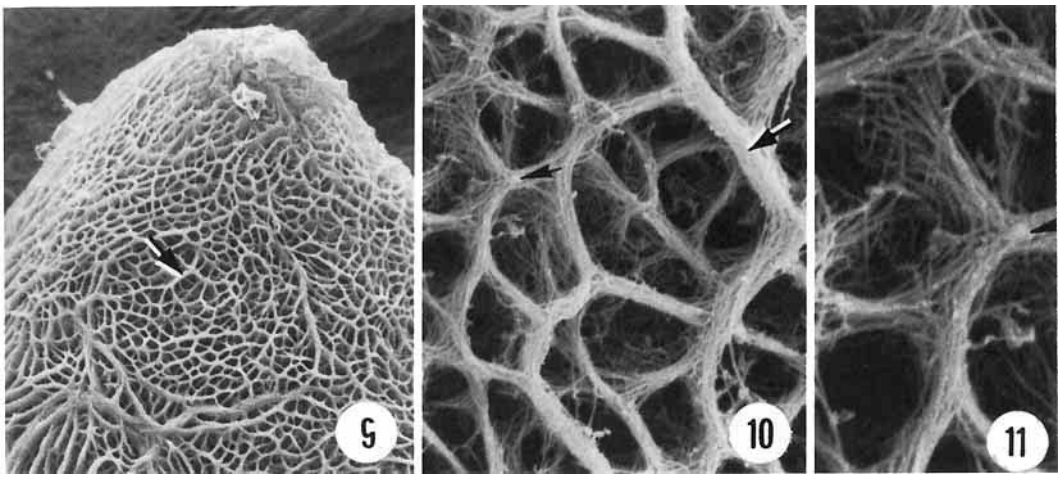


Fig. 9. SEM of a dermal papillae located on the plantar surface following degradation of the basal lamina by trypsin. The honeycomb pattern of the dermal surface is unusually well illustrated. Figures 10 and 11 are higher power magnifications (rotated ~90° to the right; note change in direction of the arrow) of the region indicated by the arrow. ×750.

Fig. 10. The foundation of dermal collagen fibrils that underlays the basal lamina is illustrated following proteolytic degradation of that structure. The fibrils are arranged in a series of variably sized, interconnected walls. The region to the left of the black arrow is illustrated at higher power in Figure 11. ×5,000.

Fig. 11. Individual dermal collagen fibrils, measuring 60–70 nm in diameter, form the underlying walls or septae that support the basal lamina. The arrangement of these collagen fibrils is responsible for the honeycomb surface appearance of dermal papillae and secondary grooves. The arrow at the right center of the micrograph indicates the same area that marked in Figure 10. ×10,000.

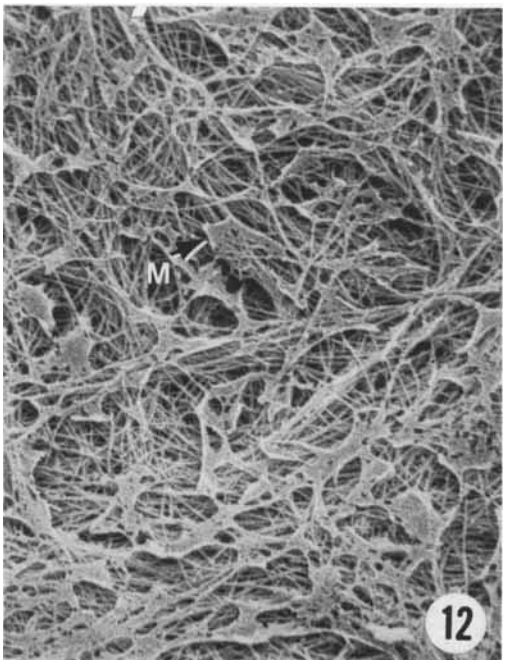


Fig. 12. A primary dermal groove is illustrated following partial proteolytic degradation of the basal lamina (a patch of the partially degraded lamina is indicated by arrow "m"). While the dermal collagen fibrils are interwoven, they do not form the high, interconnecting walls characteristic of the secondary dermal grooves or papillae. ×5,800.

lucida is not apparent [data not shown]) as opposed to that of the underlying dermal collagen fibrils—a point of some uncertainty in the work of Misumi and Akiyoshi. The epidermal basal lamina has a smooth, essentially featureless, surface appearance (see also Hull and Warfel, 1983) while the underlying dermal collagen exhibits a typical and easily discernible fibrillar pattern (Holbrook and Smith, 1981; Lillie et al., 1982). It is not clear why Misumi and Akiyoshi were unable to demonstrate a basal lamina when they used a transmission electron microscope to study samples identical to those used for SEM studies. A basal lamina is definitely present in their scanning electron micrographs and can be distinguished from the underlying collagen fibrils in their report.

The term "fiber" was applied by Misumi and Akiyoshi to the microridges depicted in our study because 1) a basal lamina could not be distingusihed and 2) paraffin sections of previously scanned dermal specimens demonstrated collagen (by the use of connective tissue stains) at the junction with the epidermis. Although the choice of the term "fiber" is unfortunate, it does emphasize the fact that complexly arranged bundles or, in many instances, interconnecting walls of collagen fibrils form the foundation upon which the epidermal basal cells deposit the basement membrane (Briggaman et al., 1971). This complex basement membrane surface which is divided and subdivided into many recesses or compartments by microridges and septae has also been described by Hull and Warfel (1983) in abdominal skin. Unfortunately, the nature of epidermal basal cell interactions with the fibroblasts of the underlying dermis that result in the formation of such a complexly folded and comparmentalized surface are unknown. Nor is this junction simply static once it is formed. Both Misumi

and Akiyoshi and Hull and Warfel describe age-dependent remodeling of the epidermal-dermal junction, although in one case the dermal surface becomes less complex while in the other it becomes more so. Remodeling of the junction of the lamina propria, a similarly complex surface, with the gingival epithelium also occurs throughout life (Loe and Karring, 1971; Klein-Szanto and Schroeder, 1977).

In the series of specimens we examined, the location-dependent, topographic features exhibited by the basal lamina-covered dermal surface did not vary with respect to location of the sample (foot, palm, or thumb) or age of the individual donor. (There were, however, differences in the form, *but not surface features*, of dermal papillae that did not correlate with either age or location.) This conservation of unique topographical regions along the dermal junction with the epidermis emphasizes the important regional differences in the function of epidermal basal cells recently reported by Lavker and Sun (1983). Studying the palmar epidermis of monkeys, these investigators demonstrated that slow cycling epidermal cells, which give rise to mitotically active ("transient amplifying cells") suprabasal cells, were located in the relatively smooth primary dermal grooves whereas epidermal basal cells ("serrated cells"—also referred to as cells with"roolets," by Horstmann [1957]) located in regions of pronounced basement membrane folding or compartmentalization were mitotically inactive and presumably had a primary function of attaching the epidermis to the dermis. The intriguing unknown element in this cell renewal system is whether the conformation of the interface (smooth or complexly folded) instructs the basal cells which role to assume. The answer to this unknown question must await demonstration of a suitable model system in which basal cells that presumably have the same potential can be placed in different regions of the epidermal-dermal junction. Similarly, the delination of those cell-cell and cell-matrix interactions that are responsible for the construction of this complex and regionally variable junction must also await development of suitable model systems.

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