AN ELECTRON MICROSCOPIC STUDY OF HUMAN EPIDERMIS*

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Since the introduction of the electron microscope there have been a series of improvements in both the microscope and in the techniques of preparing the tissue. These have resulted in its present accuracy and widespread use in the biomedical field. The electron microscope has increased resolving power attainable in sections from the previous limit of 2,000 Angstroms with the light microscope to the present limit of 20 Angstroms. In so doing, it has led to a description of many previously unseen structures, and thus to much new terminology in the field of anatomy. Many concepts on structure have had to be revised and many others have been upheld and extended by this new visual aid.

In the case of skin, the study of fine structure, or that anatomy seen with the electron microscope, has been developed over the past ten years. Most of the earlier workers were handicapped by relatively poor fixatives, embedding media and sectioning methods. These have improved remarkably in the past few years and have resulted in a clearer understanding of the fine structure of normal human epidermis (1—9).

The present work was undertaken in the hope of providing additional information on the fine structure of normal human epidermis. It was considered especially desirable to take advantage of the increased resolution attained recently, in sections using a polyester (Vestopal W) as an embedding medium.

MATERIALS AND METHODS

All normal human skin used in this study was obtained by punch biopsy from the upper arms of white persons between the ages of 46 and 67. The procedure for embedding in Vestopal W has been described elsewhere (10). In this study the RCA-EMU 2A electron microscope was used.

RESULTS

In discussing the fine structure of the human epidermis, the following subdivisions will be used: A, stratum germinativum; B, stratum spinosum; C, stratum granulosum; D, stratum lucidum and stratum corneum.

A. Stratum Germinativum. The nucleus of the basal cell is oval and has two definite membranes, an inner one approximately 300 Angstroms thick, bounded on its inner surface by many fine particles adhering so closely, that they occasionally seem to be part of the membrane, thus causing it to appear thicker than it may actually be. Numerous fine particles cover the cytoplasmic surface of the outer nuclear membrane which is separated from the inner one by a relatively constant clear area approximately 600 Å wide. In places, the outer membrane diverges from the inner one, extends deep into the cytoplasm, at times giving the appearance of forming part of the endoplasmic reticulum. Pores are seen in the outer membrane, but none have been noted in the inner one. The nucleoplasm is made up of small electron-dense particles, all approximately the same size (200 Å). The nucleolus appears to be a condensation of particles into a worm-like structure with no limiting membrane. Often several nucleoli, which may actually be portions of a single one, are seen.

The cytoplasm of the basal cell contains multiple organelles and an intricate system of vesicles and fibrils (Fig. 1). Many mitochondria are present throughout the cytoplasm, mostly encompassing and at times indenting the nucleus. The mitochondria are variable in size and shape, but average 7300 Å (2500 Å—10,000 Å) in length and 2400 Å (1500 Å—2500 Å) in width. The apparent variation in shape in sections may be due to the manner in which they have been sectioned. The mitochondria have an outer limiting membrane (75 Å), and an inner one (50 Å), the latter being folded to form several double-layered shelves or crests. These may be short or may extend as much as three-fourths of the way across the mitochondrion. The rest
Fig. 1. A view showing the relationship of the basal cells to the cells of the stratum spinosum. B, basal cell; c, centriole; cb, cell boundary; d, desmosome; er, endoplasmic reticulum; G, Golgi apparatus; m, melanin granule; M, mitochondrion; S, spinous cell; t, tonofilbril, composed of tonofilaments. Note tonofilaments in basal cell. X 11,500

Fig. 2. Irregular projection of basal cell into dermis. B, basal cell; D, dermis; v, vesicle. X 43,000
of the interior of the mitochondria is occupied by numerous fine electron-dense particles, about half the size of those noted in the cytoplasm. Also observed in most of the mitochondria are one, two, or three dense particles (Fig. 1) which are darker and larger (up to 400 Å) than those which make up the background density of the mitochondria.

The endoplasmic reticulum is relatively well developed (Fig. 1), but not as well as that noted in the spinous layer. Many vesicles, variable in size and shape, are seen throughout the cytoplasm. Some have smooth surfaces while others appear rough, due to the presence of numerous adherent RNA granules. All vesicles have a clear center with no obvious internal structure. An unusual feature of the basal cell is a number of smooth-walled vesicles often noted in irregular cytoplasmic pegs, which project into the dermis (Fig. 2). Numerous fine (200–300 Å) electron-dense particles are seen throughout the cytoplasm, and these make up the background of the cytoplasm of all epidermal cells.

Tonofilaments, either arranged into tonofibrils or lying in random fashion, are noted throughout the cytoplasm (Fig. 1). In the basal cell, the tonofilaments appear longest and most pronounced, lie perpendicular to the surface of the epidermis and attach to the inner surface of the desmosomes (Fig. 1). Each desmosome consists of a definite oval plate or thickening of adjacent portions of cell membranes, separated by what appears to be a clear area 300–600 Å wide. On occasion, in good sections and with higher magnifications, an electron-dense lamina is seen midway between both oval thickenings with an intermediate lamina located midway between the latter two lamellae. The oval thickening of each cell membrane, when viewed tangentially, i.e., from its external surface or that side facing the intermediate lamina, is about 400 Å wide and 700 Å long. This view is characterized by the presence of multiple round densities (Fig. 3), which represent the ends of the tonofibrils, attached to the cytoplasmic surface of the oval plate or thickening.

The dermal side of the basal cell is made up of irregular prolongations of the cell into the dermis, each containing, besides the vesicles mentioned, an occasional thickening of its membrane but without a similar structure apposing it (Fig. 4). Tonofibrils are attached to its cytoplasmic surface. About 300 Å below the cell membrane, another fairly definite, broader membrane (Fig. 4) runs parallel with the dermal border of the basal cell.

This membrane is made of several layers of fine fibrils. Below it is a fairly broad, less dense area containing many fine fibrils without apparent organization. Fibroblasts and bundles of collagen fibrils are occasionally seen in this region.

In the basal cell, one almost always sees melanin granules (Fig. 1). Very rarely, small particles, morphologically indistinguishable from melanin particles, are seen within mitochondria in the basal cells. The melanin granules within the basal cell are often arranged as a cap or group at the superior pole of the nucleus (Fig. 1), the location often assumed by mitochondria. The melanin granules are not, however, confined to a supranuclear zone, but may also be seen throughout the cytoplasm.

On rare occasions a centriole (Fig. 1) appears in the plane of section through a basal cell.
Fig. 4. A basal cell with projections into the dermis and the fibrillar "subepidermal membrane". B, basal cell; ct, cell wall thickening; D, dermis; sm, "subepidermal membrane". X 35,125

Fig. 5. An enlargement of the centriole and Golgi apparatus. c, centriole; G, Golgi apparatus; m, melanin granule; N, nucleus; t, tonofilaments. X 36,250
The centriole lies in a clear area (centrosome) at the superior pole, just outside the outer nuclear membrane. When cut in longitudinal section the centriole appears as would a cylinder with an outer and inner wall if sectioned longitudinally. One sees two parallel membranes approximately 0.34μ long and 0.02μ wide, separated from a similar pair by a distance of nearly 0.1μ. The region between the two pairs of membranes is somewhat denser than the surrounding cytoplasm (Fig. 5).

In the same region a Golgi apparatus is occasionally seen. In thin sections this usually consists of several smooth-walled, oval vesicles (900 Å) surrounded by numerous smaller, smooth-walled, round vesicles (500 Å) (Fig. 5).

The cell membrane between desmosomes of adjacent basal cells is for the most part straight and perpendicular to the surface of the skin. The contour, however, is interrupted by villous foldings of the cell membranes which are compressed between the two cells. In its attachment to the spinous cell, the basal cell is less regular in contour and has numerous foldings and villi between desmosomes.

B. Stratum Spinosum. Here, as in the stratum germinativum, the rounded nuclei are bounded by two membranes (Fig. 6). Nucleoli may be seen, being worm-like in shape, lacking a limiting membrane, and made up of many fine particles. Whether more than one nucleolus is ever present is difficult to determine without serial sections. No nuclear pores are present in the inner membrane. The outer membrane at times bulges away from the nucleus, often approaching the cell membrane.

The inner membrane appears wider than the outer, which is covered on its cytoplasmic side by RNA granules, giving it a rough appearance. The dimensions of the membranes are similar to those in the stratum germinativum.

Mitochondria are numerous throughout the cytoplasm, at times encircling and indenting the nucleus. Their shape and structure are similar to features seen in the basal layer. There is an intricate system of vesicles (Fig. 1) throughout the cytoplasm of the spinous cell. These vesicles are of many sizes and shapes; some are smooth and others are coated with small granules, giving a rough-walled appearance. Some smooth, double-walled vesicles are seen close to the cell membrane. These probably represent cross sections of invaginating villi from adjacent cells.

The cell membrane is quite villous, each cell held close to its neighbor by the resistant desmosomes. Between these, the membrane is thrown into irregular folds and the cell is separated from its neighbor by an “intercellular space” which is perhaps apparent rather than real. There are desmosomes in the stratum spinosum, their structure being the same as that noted in the basal layer. The cytoplasm is made up of fine electron-dense particles. No Golgi apparatus or centrioles were noted in this region of the epidermis. Tonofilaments are relatively abundant, being arranged into fibrils at their attachment to the cytoplasmic side of the desmosome and lying in a haphazard manner within the rest of the cell. The filaments were all found to be of approximately the same diameter (70–80 Å) but of varying length, probably due to a more random orientation with respect to the plane of section.

As the cell approaches the stratum granulosum, it undergoes a definite flattening with its longest diameter oriented parallel to the surface. The desmosomes are still clearly visible and retain their characteristic internal structure. The tonofilaments at this stage are arranged in a fairly regular fashion, lying horizontal rather than perpendicular to the surface.

Melanin granules are present in the stratum spinosum in small numbers. They have no visible relationship to the nucleus, in contrast to the situation in the stratum germinativum. In the higher regions of the stratum spinosum, fewer melanin granules, elements of the endoplasmic reticulum, or mitochondria are observed. In the cells of these upper spinous cell regions, however, numerous, round, smooth-surfaced, thick-walled vesicles are noted (Fig. 7). They are also found in the stratum granulosum, still retaining their shape and size, but fewer. None are seen in the stratum corneum or stratum lucidum.

C. Stratum Granulosum. In this region occurs a continuation of the process of differentiation begun in the stratum spinosum. Present, but in smaller numbers, are mitochondria, vesicles of the endoplasmic reticulum, melanin granules, and the previously mentioned smooth, thick-walled vesicles. Most characteristic, however, are the large, electron-dense keratohyalin granules (Fig. 8).

Nuclei may still be present and if seen, are irregular. If the nucleus is present the inner
membrane is still definite but the outer one is difficult to make out. An occasional nucleolus is observed and is of the same shape and structure as those previously described. Mitochondria, if present, are often perinuclear, still of the same size, but their membranes and cristae appear shrivelled (Fig. 7). The endoplasmic reticulum is not prominent.

The cell membrane has definite foldings, and retains desmosomes, which appear to be closer and more numerous than was previously noted in the deeper layers. The latter may be due to

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**FIG. 6.** A higher magnification showing the double nuclear membrane. d, desmosome; im, inner membrane; m, melanin granule; M, mitochondrion; om, outer membrane; t, tonofibril. X 24,000

**FIG. 7.** The upper stratum spinosum and the stratum granulosum. Note shape of cells. d, desmosome; kh, keratohyalin granule; M, mitochondrion; t, tonofibril (composed of filaments); tw, thick-walled vesicle. X 24,333

**FIG. 8.** The upper layers of the epidermis. d, desmosome; K, stratum corneum; kh, keratohyalin granule; SL, stratum lucidum. X 19,500
the new, somewhat crenated shape the cell has assumed, with the same surface membrane now encompassing a lesser volume. As the cell continues flattening on its move toward the surface, the keratohyalin granules seem to surround the tonofibrils as the fibrils become arranged in a horizontal, more orderly fashion.

**D. Stratum Lucidum and Stratum Corneum.**

Often visible between the stratum granulosum and stratum corneum is a layer, one cell thick, which has a moth-eaten appearance (Fig. 8). It looks as if the tonofibrils are more close to parallel with the surface and more compact, but less dense than those of the stratum corneum. When the cell passes through this region (stratum lucidum), it then assumes the homogeneous appearance characteristic of the stratum corneum (Fig. 8). In the above two cell layers, a nucleus is no longer seen. The cytoplasmic structures, including melanin, mitochondria, endoplasmic reticulum, Golgi apparatus and the smooth, thick-walled vesicles appear to be gone. The cell has become long and flat, oriented parallel with the surface. Still present are the desmosomes attaching each cell to its neighbor. The internal structure of the desmosome is vague and appears to have taken part in the process of keratinization.

The stratum lucidum is usually one cell thick, while the stratum corneum is about three to five cells thick, depending on the region from which the tissue was obtained. The outer cells (stratum disjunctum) fall free only when the desmosomes break, this being the last step in the progression of the cell.

**DISCUSSION**

The normal human epidermis appears to be delimited from the dermis by a membrane, fibrillar in nature, which runs parallel with and adjoins the underside of the epidermis. It is conceivable that this membrane plays a part in protecting the epidermal cells from invasion from below and likewise protects the dermis against invasion from above. The possibility that it functions as a structure in the cohesion between epidermis and dermis is also likely. The basal cells send multiple projections into the dermis. These, too, appear to play a part in attaching the epidermis to the dermis. Vesicles are often seen in these pegs and as Odland (5) has suggested, it is possible that they play a part in the transfer of materials to and from the epidermis. As mentioned, double-walled vesicles have been noted just inside the cell membrane as if a villous process from one cell had been projected into the cytoplasm of its neighbor and then pinched off.

The basal cells seemingly have relatively straight lateral borders and are attached to one another by the desmosomes. Thus, one must conclude that in essence the basal cell is a type of prickle cell. Though the borders appear straight, numerous foldings of the cell membrane are squeezed against each adjacent cell. This occurs between desmosomes and is due to the closeness of neighboring cells. The reason the “prickles” are not as obvious here as they are in the spinous layer is that the basal cells are compressed against each other so tightly that cell separation does not occur.

In the basal layer numerous and regularly oriented tonofibrils lie perpendicular to the surface of the skin. As the cell moves toward the stratum corneum these tonofibrils, which do not pass from cell to cell, become arranged parallel to the surface, and most likely play an integral role in the process of keratinization. Keratohyalin granules are easily visualized in the stratum granulosum as electron-dense, homogeneous, stellate structures which Selby (3) feels might be cellular debris. As the cell is changing shape the tonofibrils change from a perpendicular to a horizontal position, moving closer together, and enmeshing the keratohyalin granules.

The force holding together all epidermal cells is the desmosome. The desmosomes are closer together in the stratum granulosum, due to the new shape of the cell. These remain intact throughout the passage of the cell from the basal cell layer to the surface, and tend to undergo keratinization with the rest of the cell. When they finally break, the cell is shed. Desmosomes are noted to hold firm against the melanocyte processes, which appear to force the epidermal cells apart, and yet are unable to break the desmosomes. Alterations in the integrity of the desmosome very probably play a large part in diseases that cause increased or decreased shedding as well as those resulting in vesiculation.

As the cell continues its movement upward, the vital cytoplasmic organelles begin to decrease in number, probably due to the decreased activity of the cell and its beginning degeneration. A
structure first appearing in great numbers in the upper spinous layer is the smooth, thick-walled vesicle. Selby (3) and Odland (9) feel that the internal structure of these vesicles is sufficiently well organized to warrant considering them as unusual forms of mitochondria. Fewer of these are noted in the stratum granulosum and none in the stratum lucidum and stratum corneum.

At the lower level of the stratum corneum a moth-eaten-appearing cell layer is noted. This layer, easily recognizable in electron micrographs, most likely represents the stratum lucidum, which often is not visible with the light microscope, but in essence is always present. The stratum corneum varies in thickness depending on the region from which the tissue was taken.

SUMMARY

1. Observations of the fine structure of normal human epidermis are reported.
2. A polyester, allowing increased resolution, was used as an embedding medium in this study.
3. The fibrillar structure of the "subepidermal membrane" and its relationship to the epidermis as well as the contour, cytoplasmic structures and organization of the tonofibrils of the epidermal cells are described. The changes that occur in the cell contour and its cytoplasmic organelles as it progresses through the stratum corneum and the consistently present stratum lucidum are discussed.

REFERENCES