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## OBSERVATIONS ON THE ULTRASTRUCTURE OF HUMAN SKIN\*

FUNAN HU, M.D.\*\* AND ROBERT R. CARDELL, JR.\*\*\*

### INTRODUCTION

THE DEVELOPMENT of the electron microscope and techniques related to biological and medical electron microscopy has provided a method for extending and refining our knowledge of histology. Human skin has received considerable attention as an object of electron microscopy studies<sup>1-19</sup> and the ultrastructure of human skin continues to be of interest. In an attempt to study various diseases of the skin with the electron microscope, the authors considered it necessary to have more information on the ultrastructure of normal skin. Therefore it is the purpose of the present report to describe, as completely as possible, the organization of human skin as revealed by the electron microscope. Since the initiation of this study several communications have appeared on the ultrastructure of skin;<sup>20-24</sup> these will be discussed in light of our observations and interpretations.

### MATERIALS AND METHODS

All human skin used in this study was obtained from the medial aspect of the lower arms of both Caucasian and Negro volunteers. The specimen was immediately fixed in 1 per cent buffered (veronal-acetate) osmium tetroxide (adjusted to pH. 7.4)<sup>25</sup> and cut into small pieces less than one cubic millimeter. The tissue was transferred to fresh fixative and allowed to remain in the fixative for four hours with occasional shaking. The specimen was washed thoroughly with 50 per cent stock buffer solution to remove the excess osmium tetroxide and dehydrated with a graded series of acetone until the tissue was placed in 100 per cent acetone. The acetone was replaced by absolute alcohol in order to stain the tissue with phosphotungstic acid, which was achieved by immersing the specimen in a 1 per cent solution of phosphotungstic acid in absolute alcohol for one hour. The alcohol was replaced by 100 per cent acetone which had been dried over  $\text{Cu SO}_4$  and the infiltration of the embedding medium was begun. As pointed out in a previous communication<sup>26</sup> vestopal W. was found to be a superior embedding material for skin. The conclusion that vestopal W. preserves the cellular detail of human skin better than other embedding media is shared by Zelikson and Hartmann.<sup>17</sup>

The ultrathin sections were cut on an LKB ultratome and the sections having a gray to silvery appearance were placed on parlodian-carbon coated Athene #100 grids.<sup>27</sup> The sections were viewed with a modified RCA EMU2B electron microscope and electronmicrographs were taken of selected areas.

### OBSERVATIONS

#### LIGHT MICROSCOPY

For purpose of orientation, a description of the structure of human skin as revealed by the light microscope is included. Figure 1 is a photomicrograph of

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a skin section stained with hematoxylin and eosin and illustrates the epidermis and upper half of the dermis of the skin.

The epidermis may be divided into four cell layers. Describing them from the outer surface of the skin toward the dermis, they are: (1) Stratum Corneum (the horny layer); (2) Stratum Granulosum (the granular cell layer); (3) Stratum Malpighii (stratum spinosum or the prickle cell layer) and lastly; (4) Stratum

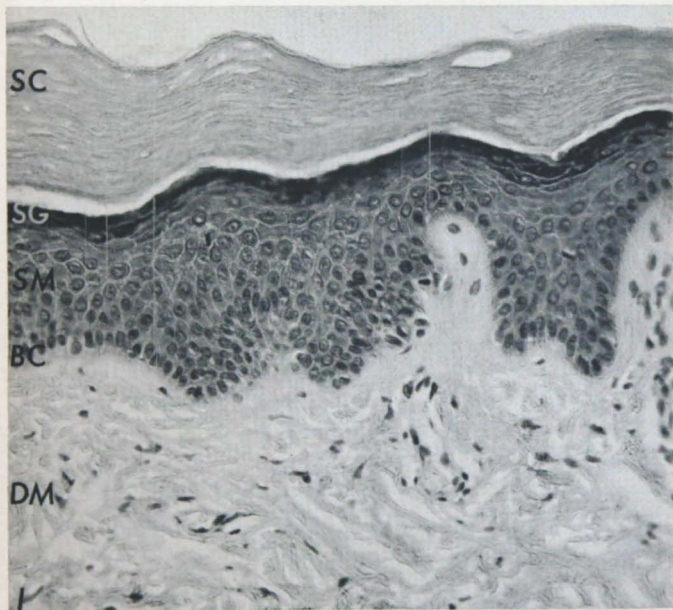


Figure 1

A section of human skin stained with hematoxylin and eosin. The various layers of skin are shown: St. Corneum (SC), St. Granulosum (SG), St. Malpighii (SM), St. Germinativum (BC) and dermis (DM).

Figure 2

A section through the dermis of skin. Longitudinal and cross sections of collagen fibers (cf) are shown. Note the portions of the fibroblasts (f). Magnification 16,000 x).

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Germinativum (the basal layer). In skin from the palms and soles there is an additional layer, the stratum lucidum, which is located above the granular cell layer and beneath the horny layer. This layer when present is composed of a few layers of non-nucleated homogeneous material and has a somewhat transparent appearance. The stratum corneum consists of layers of horizontally oriented fibrous protein, or keratin, and is also non-nucleated. The cells of the granular layer are more or less diamond-shaped with their axis oriented parallel to the skin surface. They are filled with coarse granules which are irregular in shape and are known as keratohyalin granules. The cells in the stratum Malpighii are polyhedral in shape and form a mosaic-like pattern. They are separated by spaces which are traversed by intercellular bridges.

Two types of cells, the basal cells and the melanocytes, are found in the basal layer. The basal cells are columnar in shape and lie with their long axis vertical to the skin surface. They have an oval or elongated nucleus which is also oriented perpendicularly to the skin surface. In pigmented skin, these cells have been shown to contain melanin granules which are often concentrated around the upper pole of the nucleus to form the so called supranuclear caps. Basal cells are united to one another and to the overlying prickle cells by intercellular bridges.

### Figure 3

Fibroblast in the dermis. The oval nucleus (n) is shown located in the fibrillar cytoplasm (fb). Also shown in the cytoplasm are the mitochondria (m) and the electron-dense granules (g). Observe the intimate relationship of the "arms" of the fibroblast to the collagen fibers. (Magnification 11,000 x).



In routine hematoxylin and eosin-stained sections the melanocytes (clear cells of Masson or dendritic cells) appear as small cells with a dark nucleus and clear cytoplasm. They are located between epithelial cells of the basal layer. In sections of darkly pigmented skin stained with silver or DOPA reagents, the melanocytes appear as dendritic cells with numerous long branching processes.

Although routine stains do not show a basement membrane, this structure may be demonstrated by employing the Hotchkiss-McManus periodic acid-Schiff stain.<sup>29</sup> The basement membrane, which is PAS positive, appears as a thin, homogeneous, dense band at the dermo-epidermal junction. This membrane separates the epidermal cells from the fibrous connective tissue of the dermis, and the PAS positive reaction is attributed to the presence of polysaccharide material in this zone.<sup>29</sup>

The dermis is the fibrous layer of the skin, with three types of fibers occurring: collagenous, elastic, and reticulum fibers. Of these, collagen is the major fibrous component of the dermis. The other two types of fibers are seen only by special staining procedures.<sup>30</sup>

#### ELECTRON MICROSCOPY

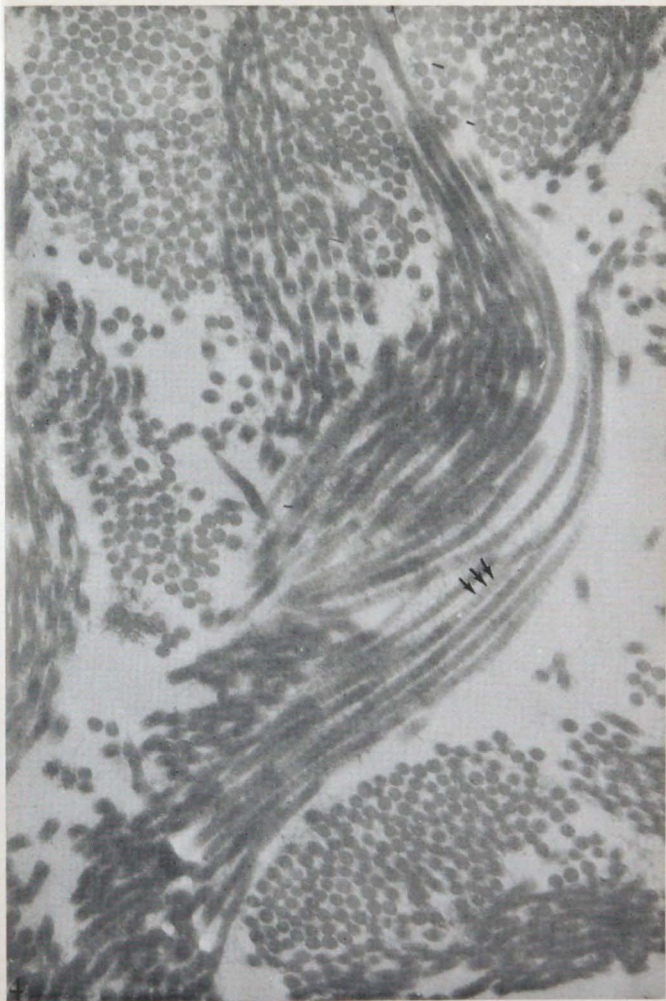
##### *Dermis*

The dermis is of variable thickness consisting of bundles of collagen oriented in various directions and fibroblasts scattered among the collagen bundles (Figure 2). The fibroblast is a stellate cell with the "arms" of the cytoplasm appearing to encircle the bundles of collagen (Figure 3). The fibroblast is bounded by a plasma

#### Figure 4

A higher magnification of collagen fibers. Cross and longitudinal sections are shown. Note the periodicity of the collagen fibers (arrows). (Magnification 45,000 x).

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membrane and the cytoplasm contains the usual cytoplasmic organelles: mitochondria, endoplasmic reticulum, ribosomes attached to endoplasmic reticulum, free ribosomes, and some unidentified electron-dense granules (Figure 3). In addition, the cytoplasm of the fibroblast has many fine fibrils distributed throughout the cell (Figure 3). The oblong nucleus has a double membrane (nuclear envelope) and occasionally nucleoli are seen within the nucleus. The nuclear structure of the skin cells is not unlike that described by other workers<sup>8,20</sup> and we shall not refer to the ultrastructure of these nuclei except to note the changes in shape within the various cells.

The collagen fibers are found in bundles of approximately 350 fibers per bundle. The fiber is approximately 78  $m\mu$  in diameter and the distance between the main period bands is 59  $m\mu$ . (Figure 4).

#### *Basement Membrane*

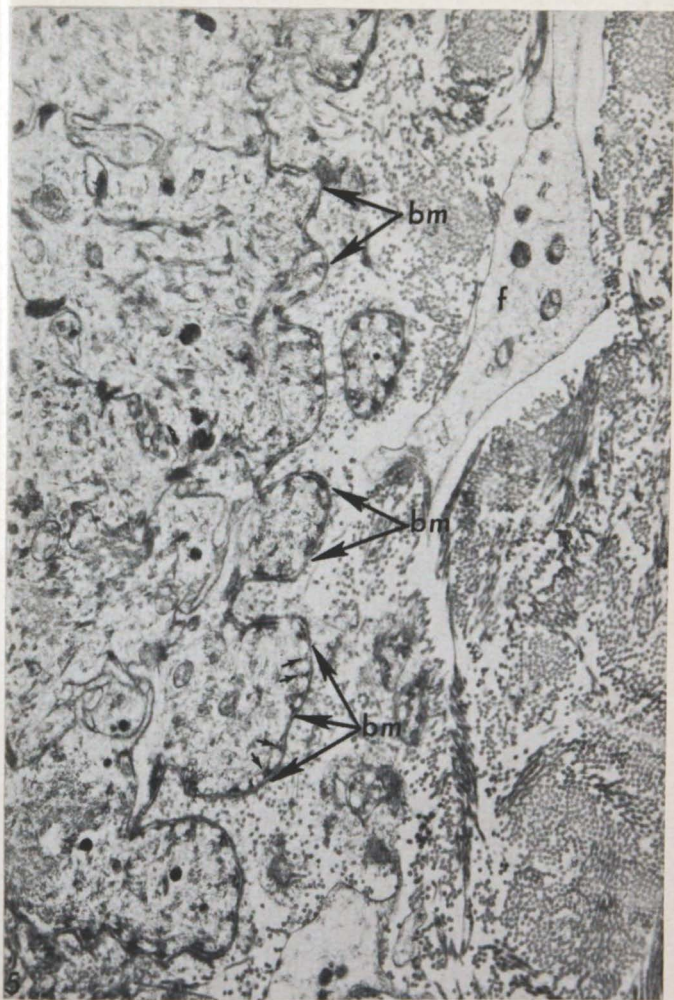
The separation of the dermis from the epidermis is effected by a membrane which follows the contour of the epidermis (Figures 5, 6). This membrane is approximately 35  $m\mu$  thick (Figure 6) and is identical to the structure termed "dermal membrane" by Sebly.<sup>2</sup> Adjacent to the basement membrane, the plasma membrane of the basal cells has electron-dense areas to which the tonofibrils attach (Figure 6).

#### *Epidermis*

*Stratum Germinativum:* This layer of cells usually has cells oriented with their long axis perpendicular to the basement membrane (Figure 7). The nucleus of these cells is oblong, and the cytoplasm has a fibrous appearance due to the presence of a large number of tonofilaments. The tonofilaments come together to form

#### Figure 5

The dermo-epidermal junction. The epidermis is shown on the left and the dermis on the right, separated by the basement membrane (bm). A series of arrows indicates the location of the electron-dense areas on the plasma membrane of the basal cells. (Magnification 16,000  $\times$ ).



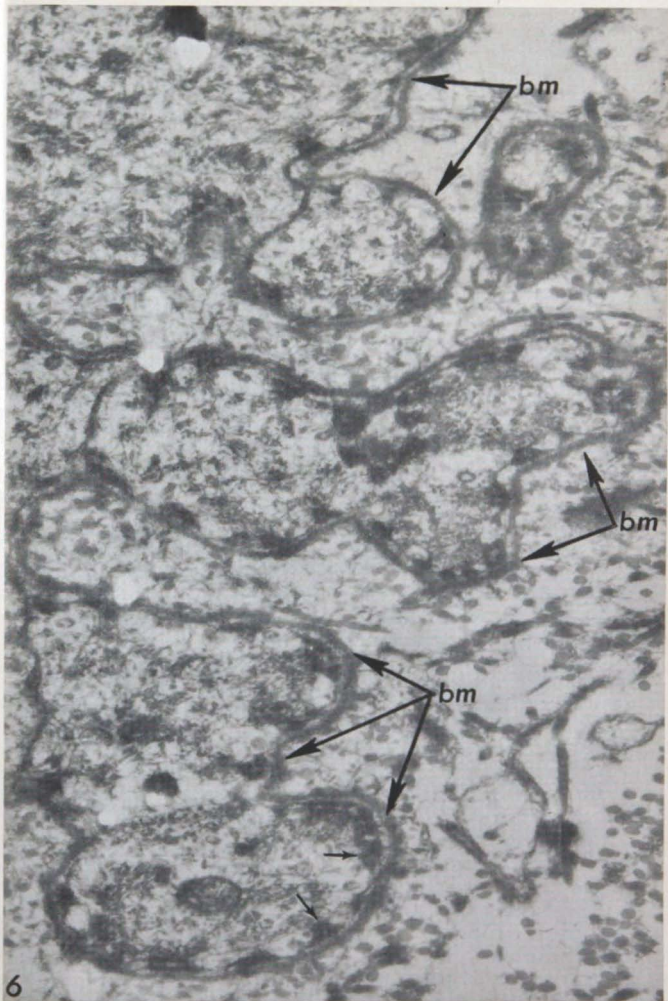
tonofibrils which make up a part of the desmosome. Mitochondria are found throughout the cytoplasm but they tend to be concentrated near the nucleus (Figure 7). Melanin granules of irregular shape are very abundant in the basal cell of the pigmented skin, usually occupying a supranuclear position; however, they are found also in the perinuclear position (Figure 7). The dermal side of the basal cell has many irregular projections into the dermis which cause the dermo-epidermal junction to be very irregular (Figures 5, 6). The cell membrane between the basal cells are folded considerably giving the impression that the cells are compressed together. Desmosomes occur between the basal cells (Figure 7).

Occasionally one observes a cell, triangular in shape, with the long axis of the nucleus parallel to the basement membrane and with melanin granules scattered evenly throughout the cytoplasm. This cell is termed a "peg cell."<sup>12</sup> It is not known if this actually represents a different functional cell type or the appearance of this cell is a result of a particular angle through which the cell was cut. Charles and Ingram<sup>14</sup> have described triangular-shaped cells between the cells of the basal layer. They interpreted these cells to be "amelanotic melanocytes". They noted the enmeshing of the collagen fibrils in the dermis around the melanocyte's cell membrane and differentiated the melanocyte from the basal cell by the lack of dense areas on the plasma membrane of the melanocyte.

*Stratum Malpighii*: The most striking characteristic of the cells in this group is the irregular and highly folded cell membranes (Figure 8). Usually at the folds are located the desmosomes (Figure 9) between adjacent prickle cells. There are also desmosomes between the prickle cells and the basal cells, and between the prickle

Figure 6

A higher magnification of the basement membrane. (Magnification 45,000 x).



cells and the granular cells. The desmosome results from an aggregation of tonofibrils attaching to the cell membrane at a particular point and a similar situation occurring in the adjacent cell (Figure 9). The tonofilaments do not go through the cell membranes but stop at each cell membrane, thus leaving a space between the cell membranes in which there are no tonofilaments. The cytoplasm of the prickle cell possesses many tonofilaments oriented in various directions and numerous mitochondria scattered throughout. The nucleus is oblong with the long axis at right angles to the long axis of the basal cell nuclei or parallel to the surface of the skin.

*Stratum Granulosum:* The cells of this layer are very elongated and narrow with their long axis oriented parallel to the surface of the skin. The nucleus is much more elongated than the nucleus of the prickle cell. The cytoplasm contains the usual components, but the mitochondria appear to be more evenly distributed throughout the cytoplasm rather than being concentrated around the nucleus (Figure 10). The desmosomes are still present, but appear to be more prominent on the side of the cells adjoining the prickle cells than between the granular cells and the stratum corneum (Figure 10). The most striking characteristic of these cells is the appearance of the irregular shaped electron-dense keratohyalin granules (Figure 11). The granules become more concentrated in the cells nearer the stratum corneum. These granules are evenly distributed throughout the cytoplasm of the granular cells.

*Stratum Corneum:* The stratum corneum varies in thickness according to the region from which the skin specimen was obtained. From Figure 12 it is obvious

#### Figure 7

The basal cells of the epidermis. The basement membrane (bm) runs along the bottom of the electronmicrograph. The basal cells are shown as columnar-shaped cells with melanin granules (mg) appearing as electron-dense bodies in the supra-nuclear position. (Magnification 11,000 x).

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that these are highly modified cells, possessing neither nucleus nor cytoplasmic constituents such as mitochondria, Golgi apparatus, endoplasmic reticulum, etc. Indeed, these cell layers have been characterized by some workers<sup>20</sup> as being "homogeneous";\* but Figure 12 reveals that there is present in the cells of the stratum corneum a network of fine fibrils which appear to be oriented tangentially to the surface of the skin. It is believed that these fibrils represent the final transformation state of the tonofibrils of the basal and prickle cells.

\*Zelickson,<sup>24</sup> in a later communication, also described the presence of filaments in this region.

#### DISCUSSION

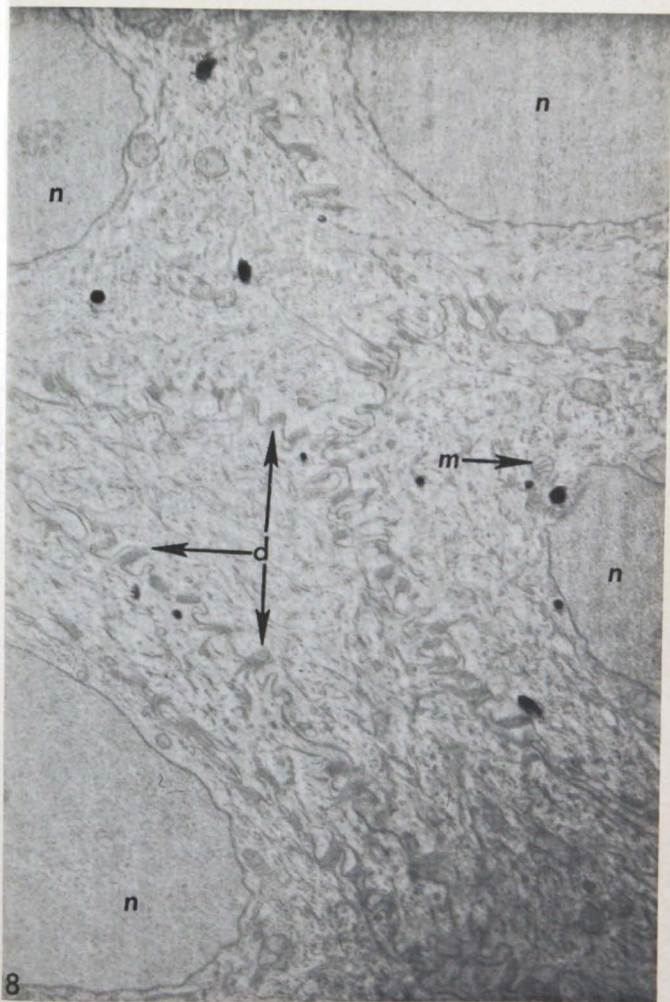
One of the main functions of skin is to serve as a protective envelope of the body. Two products of the epidermis, the keratin and the melanin pigment, are manufactured by the epithelial cells, or the keratinocytes<sup>31</sup> and the melanocytes, respectively; and play an important role in the fulfillment of this primary function.

The process of keratinization involves the transformation of living epithelial cells into dead cornified material, a fibrous protein, the keratin. Brody<sup>32</sup> in an excellent study of the horny layer of the skin of the guinea pig with the electron microscope, divided the horny layer into three sublayers: basal, intermediate, and superficial. The division of these three sub-layers was based on the distribution of the characteristic keratin pattern, the appearance of the cell boundaries, and the intercellular spaces. The cells of the horny layer do not form any syncytium, and it is a two-component system, consisting of filaments and the amorphous interfila-

#### Figure 8

A section through five prickle cells. The serrated appearance of the cell boundaries and the location of the desmosomes (d) are shown. (Magnification 13,000 x).

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mentous mass in which they are embedded. The cells show broad cell boundaries and are separated by a distinct intercellular space. The electronmicrographs of the horny layer of human skin in our study reveal a network of fine fibrils which are not unlike the characteristic keratin pattern described by Brody in the guinea pig skin. The tubular or non-tubular nature of the fibrils was not ascertained in our study of human skin. It is evident, however, that there is a difference in the staining reaction in this area as shown by the dark, presumably the interfilamentous substance, in contrast to the lightly or unstained keratin fibrils, or "tubules" as suggested by Brody. The cells in the horny layer of human skin, like those in the guinea pig skin, possess broad cell boundaries. The cell boundary consists of a fairly opaque, broad, inner zone and an opaque, fine, outer membrane. In a recent paper Zelickson<sup>24</sup> has not been able to show in the stratum corneum of human epidermis the three sublayers Brody described in the guinea pig skin. However, he did concur with Brody that the horny cells are formed by a binding together of filaments in an electron-dense cement substance which is presumably derived from the keratohyalin granules.

Based on his observations Brody<sup>32-35</sup> advanced a hypothesis according to which the keratin is formed from tonofilaments and keratohyalin granule material, partly through a gradual incorporation of tonofilaments into the keratohyalin granules. The specific keratohyalin material would then form the interfilamentous component. This hypothesis is supported by the fact that tonofibrils show the same x-ray alpha-

#### Figure 9

A higher magnification of the desmosomes. Note the convergence of the tonofibrils from the cell cytoplasm into the desmosomes. (Magnification 30,000  $\times$ ).

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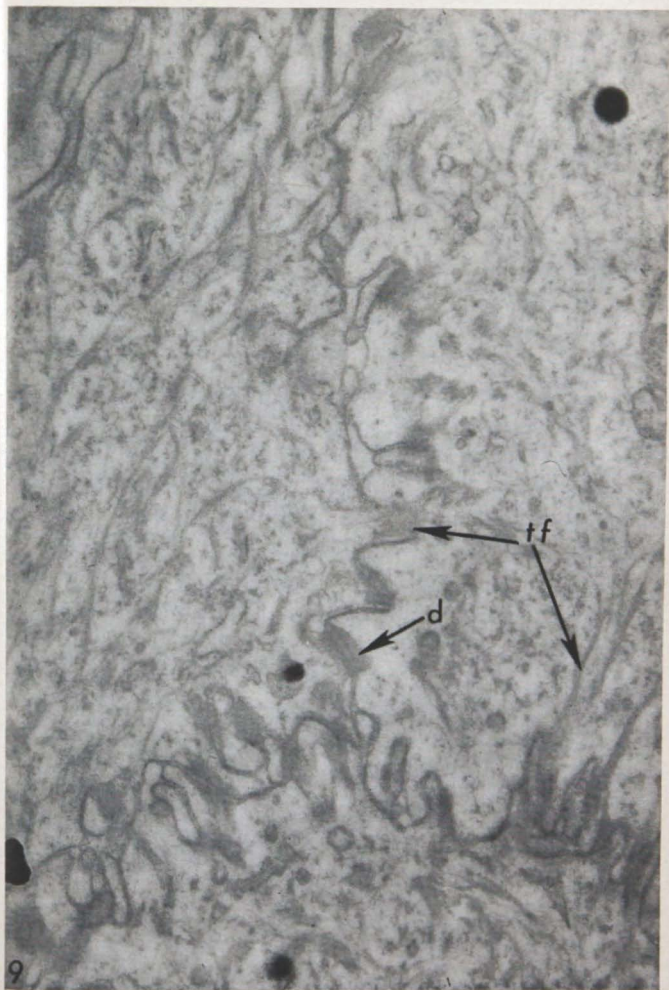


diagram as do keratin fibers<sup>34</sup> and that both tonofilaments and keratin filaments have a diameter of 100 angstrom units.<sup>34</sup> Histochemical observations also suggest that tonofibrils are the crystallization center of keratin formation.<sup>36</sup>

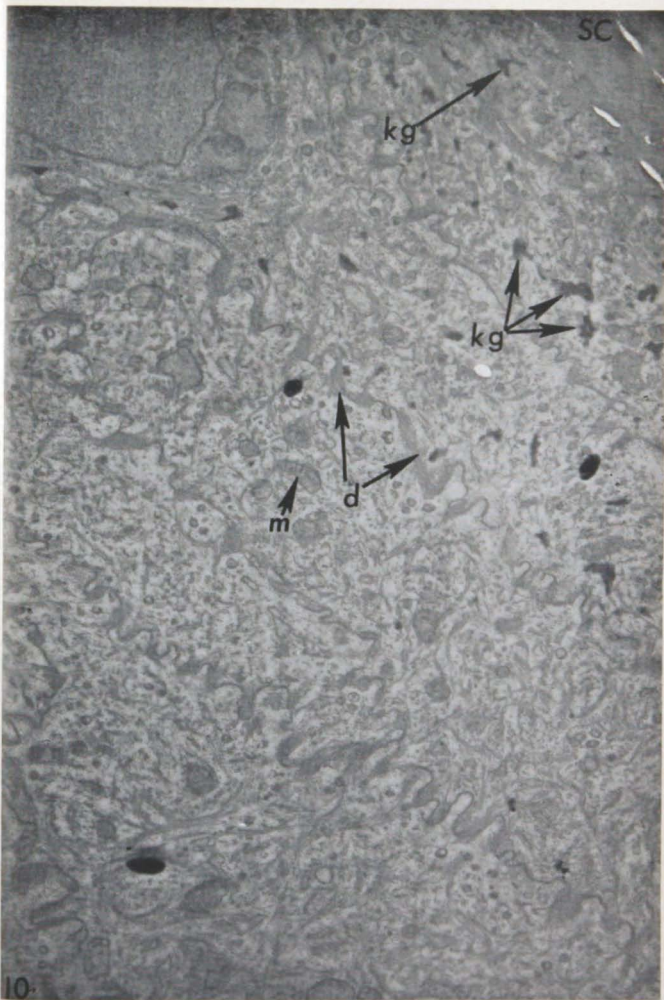
Birbeck and Mercer<sup>37</sup> observed a similar structural pattern in the keratogenous zone of the human hair follicle. They interpreted the less opaque component as filaments constituting the fibrous protein of the keratin, the alpha-keratose. The opaque interfilamentous material was considered to consist of an amorphous protein corresponding to gamma-keratose.

The other cell system in the epidermis is the melanocytes which form a final product, the melanin. The melanocytes are shown by electron microscopy<sup>22</sup> to be characterized by the following features: clear cytoplasm, melanin granules in varying stages of maturity, a well developed Golgi region, absence of desmosomes, a few small mitochondria, and a smooth nuclear membrane. In our studies we have not been able to distinguish a cell which possessed enough significant differences from other cells in the basal layer to merit terming the cell a "melanocyte".

Between the basal cell layer of the epidermis and the collagen of the dermis is a membrane which has been termed "basement membrane" by several authors<sup>5,11,20,23,38</sup> and "dermal membrane" by Selby.<sup>7</sup> Although the term "basement membrane" as employed by light microscopist is not identical to the structure termed "basement membrane" by electron microscopists, the authors have decided to use the term

Figure 10

A section through the region of *St. Malpighii* and *St. cornuem* (SC) showing the orientation of the cells of *St. granulosum*. Note the keratohyalin granules (kg) in the granular cells. (Magnification 16,000 x).



10.

"basement membrane" for the thin membrane which separates the dermis from the epidermis in human skin. This membrane is undoubtedly homologous to the membrane which surrounds capillaries,<sup>39,40</sup> and this membrane has also been termed a "basement membrane". Perhaps after more study concerning the occurrence, structure, and function of the various types of membranes which have been referred to as "basement membrane", a system of terminology which clearly distinguishes these very different structures may be devised.

The dermis represents a fibrous layer of the skin with three types of fibers occurring: collagenous, elastic and reticulum fibers. Among these, collagen forms more than 98 per cent of the connective tissue of the dermis.

The close association of the collagen fibers with the fibroblasts is apparently related to the function of the fibroblast e.g., to produce collagen. No evidence has been produced which indicates that collagen synthesis occurs within the fibroblast, but rather the collagen fiber seems to form adjacent to the cell membrane. Indeed, it has been shown that the fibroblasts become reduced to thin protoplasmic sheets at the periphery of the cell and they appear as little more than two parallel plasma membranes. In one instance such a thread-like extension exhibited a periodicity comparable to that of collagen.<sup>41</sup>

The ultrastructural organization of the fibroblast in the dermis of the human skin which we have studied does not appear to be involved in active collagen synthesis. There is not an abundance of endoplasmic reticulum present nor do we find the "characteristic vesicles" which Mercer<sup>42</sup> emphasized as a component in the cytoplasm

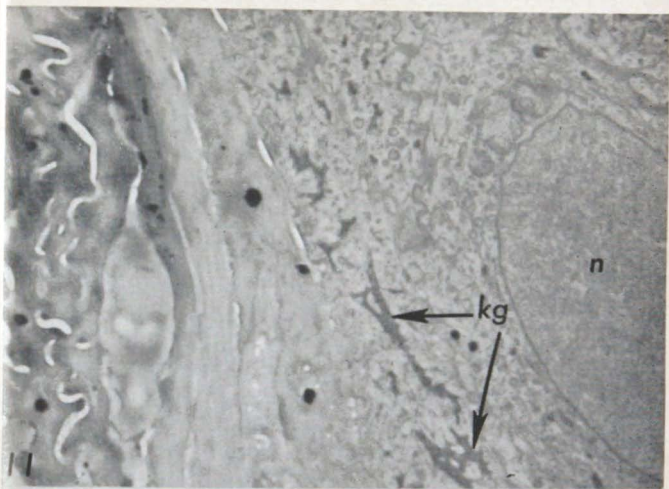
Figure 11

A section showing the junction of *St. corneum* and *St. granulosum*. Note the various layers of *St. corneum* and the keratohyalin granules. (Magnification 13,000 x).

Figure 12

A higher magnification of *St. corneum*. The fibrillar appearance of the horny layer is shown. (Magnification 13,000 x).

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of the fibroblast. However, the cytoplasm of the fibroblast does contain many fine fibrils which may be related to the synthesis of collagen.

Since elastic tissue constitutes only a small percentage of the fibers in the normal dermis, it has not been described extensively in electron microscopic examination. It appears that the elastic fibers have no well defined structural characteristics except that they are fibrillar in nature.<sup>43</sup> The first electron microscopic examination of thin-sectioned elastic fibers according to Charles,<sup>44</sup> appears to be that of Bahr and Engström.<sup>45</sup> The fibers shown by them in cross section were homogeneous, except for a more electron-dense boundary. Linden et al.<sup>46</sup> examined sections of human skin and showed spirally twisted fibrils which they considered to be elastic tissue. We have not seen any fibers in the dermis which we could characterize as elastic fibers.

#### SUMMARY

Figure 13 is a diagrammatic representation of the ultrastructural organization of a section through human skin. The skin is composed of the dermis with bundles of collagen randomly oriented and intimately related to the fibroblast. Details of the ultrastructure of collagen and the fibroblasts are presented and some information on collagen synthesis is discussed. A basement membrane is described between the dermis and epidermis. The ultrastructure of the various epidermal cells is presented and a description of distinguishing characteristics for each cell type is described. The presence of fibrils within the stratum corneum is clearly shown.

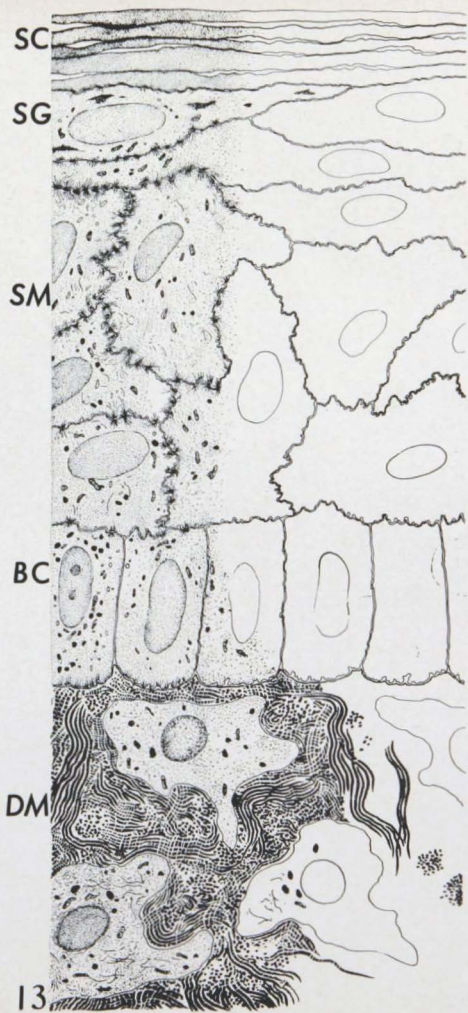
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#### Figure 13

A diagrammatic representation of the layers of skin. St. corneum (SC), St. granulosum (SG), St. Malpighii (SM), St. germinativum (BC) and dermis (DM).

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